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Observations on Sexual Reproduction in a *Chlamydomonas**

by Yoshihiro Tsubo**

小 倉 謙 博 士 記 念 号
第 1 部

Ogura Commemoration Number
Part 1

昭和 30 年 10 月 14 日広島における総会において，日本植物学会は，小倉謙博士が昭和 21 年より 30 年にわたり会長として誠意をもって多年本会のために尽された功績を記念し，あわせて博士の還暦に祝意を表するため，昭和 31 年秋に記念号を出版することになりました。このため友人門下から寄せられた多数の論文を，植物学雑誌の本号及び次号に「小倉謙博士記念号」として編集しました。

At the general meeting held at Hiroshima on October 14, 1955, the Botanical Society of Japan has decided to issue the special number in autumn of 1956 commemorating Dr. Yudzuru Ogura's long and earnest service to our Society as the president from 1946 to 1955 and celebrating his sexagenary birthday. Many articles have been contributed by the friends and pupils of Dr. Ogura, and they are compiled in the present and the next numbers of the Botanical Magazine (Tokyo) as 'Ogura Commemoration Number'.

Heterothallic strains of a *Chlamydomonas* sp. were isolated in 1952 from a field near Kobe city, Hyogo prefecture, Japan. Before starting experimental work with this organism, it was necessary to study some of the factors for gamete-production: such studies have been made for other species[16]. The following observations relates specifically to the nutritional control of gamete-formation and copulation in *Chlamydomonas* sp. 24.

Morphology

The cells are broadly ellipsoidal in shape. The central nucleus is surrounded by a basal cup-shaped chloroplast with a round pyrenoid at one side. A single stigma is seen at the front. Two flagella are 1–1.5 times the length of the cell body, inserting at a wedge-shaped structure of the cell wall at the vegetative cells are *ca* 15 × 10μ (Fig. 1, A). Under suitable conditions copulating gametes attach at first by their anterior papillae at right angles (Fig. 1, I, J, K). A protoplasmic bridge can be seen between the copulants (Fig. 1, J) just like those reported by Lewin and Meinhart in *Chl. moewusii*. These gametes ultimately fuse after withdrawing their flagella (Fig. 1, L–P), and form a round zygote (Fig. 1, Q–T). A thick verrucose zygote wall is ultimately formed beneath the primary zygote membrane (Fig. 1, Q–T), which is stained specifically with Schiff's reagent as observed in *Chl. moewusii* by Lewin*. The verrucae are always endowed with cell walls which are characteristic of cellulose. A mature zygote freed from the primary membrane is green, and measures 10–15μ in diameter (Fig. 1, T). It takes at least a week for maturation.

Observations on Sexual Reproduction in a *Chlamydomonas**

by Yoshihiro Tsubo**

坪　由　宏：　クラミドモーナスの有性生殖についての観察

Heterothallic strains of a unicellular green alga, *Chlamydomcnas sp. 24*,*** were isolated in 1952 by the acetone method[5] from soil of a rice field at Sasayama, Hyogo prefecture, Japan. Before genetic investigations could be carried out with this organism, it was necessary to study some of the factors controlling sexual reproduction: such studies have been made for other species of *Chlamydomonas*[8, 10, 15, 16]. The following observations relates specifically to the nutritional control of gamete-formation and copulation in *Chlamydomonas sp. 24*.

Morphology

The cells are broadly ellipsoidal or subspherical (Fig. 1, A–T). The central nucleus is surrounded by a massive cup-shaped chloroplast, containing a round pyrenoid at one side. A rod-shaped stigma is present at the equatorial level. Two flagella are 1–1.5 times as long as the cell and born at the apical end, originating at a wedge-shaped papilla, beneath which lie two contractile vacuoles. Vegetative cells are *ca* $15\mu \times 10\mu$ (Fig. 1, A, B), and gametes *ca* $7\mu \times 5\mu$ (Fig. 1, F, G). Copulating gametes mate in " vis-à-vis " pairs, with their wedge-shaped papillae at right angles (Fig. 1, J–L): a protoplasmic bridge can be seen between the copulants (Fig. 1, J) just like those reported by Lewin and Meinhart in *Chl. moewusii*[9]. These gametes ultimately fuse after withdrawing their flagella (Fig. 1, L–P), and form a round zygote (Fig. 1, Q–T). A thick verrucose zogote wall is ultimately formed beneath the primary zygote membrane (Fig. 1, Q–T), which is stained specifically with Schiff's reagent as observed in *Chl. moewusii* by Lewin[6]. The swarmers are always endowed with cell walls which are characteristic of cellulose. A mature zygote freed from the primary membrane is green, and measures 10–15 μ in diameter (Fig. 1, T). It takes at least a week for maturation.
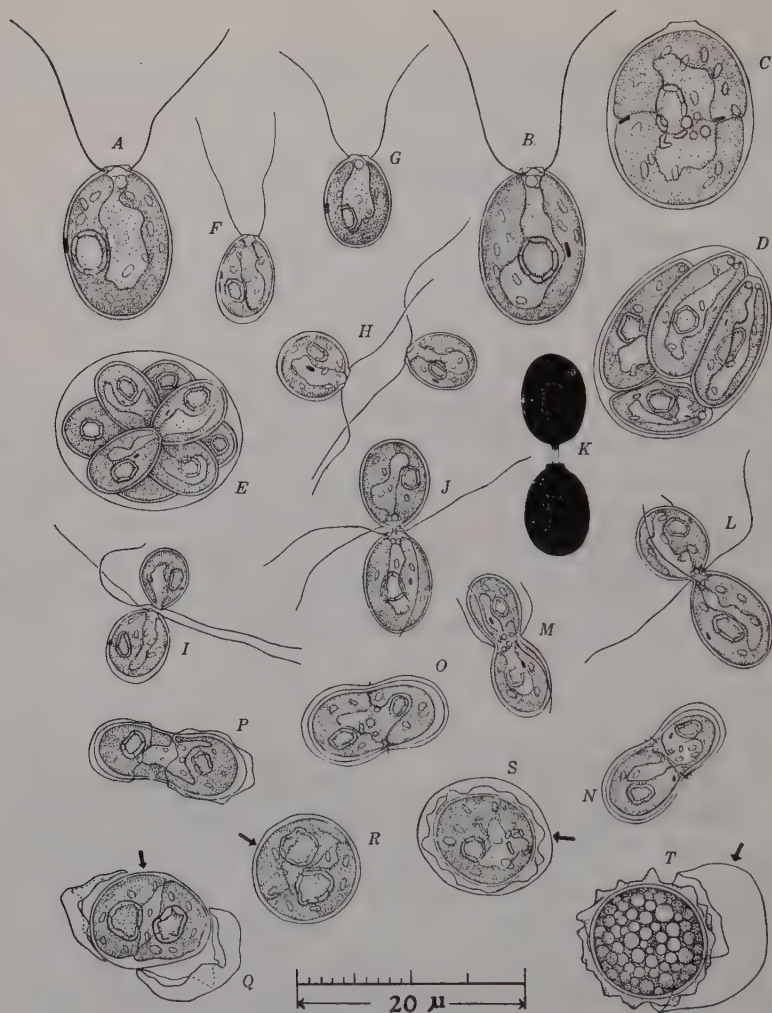
Fig. 1. *Chlamydomonas sp. 24* (×1500)

A, B; Vegetative cells: C-E; Dividing stages: C; An early stage of cell division: D; Division into 4 cells: E; Division into 8 cells: F, G; Gamete cells: H-L; Mating of gametes, a protoplasmic bridge can be seen between the copulants (J): K; A schematic representation of a "vis-à-vis" pair at a right angle with wedge-shaped papillae; Flagella are not drawn: M-T; Maturation of zygote; membrane indicated with ↑ in Q-T is the primary zygote membrane.

Reproductive Differentiation

This alga can be grown under constant illumination from fluorescent tubes (1500-2000 lux) at *ca* 23°C. in the following medium (pH 6.5).

Composition of medium (mg/1000 ml, dist. water):

NH_4NO_3, 400; $MgSO_4.7H_2O$, 100; KH_2PO_4, 100; $CaCl_2$, 50;

$FeSO_4.7H_2O$, 10; $CoCl_2.6H_2O$, 0.50; $MnCl_2.4H_2O$, 0.39;

H_3BO_3, 0.60 ; $ZnSO_4.7H_2O$, 0.06 ; $CuSO_4.5H_2O$, 0.006 ;
$H_3PO_3.12MoO_3.6H_2O$, 0.003 ; Sodium Citrate, 200.
Solid media are prepared with 1.0–1.5% agar.

The present species is isogamous and heterothallic. Cells of opposite mating
types, when transferred from agar slant cultures into liquid medium, become motile
in a few minutes, but no copulation can be observed immediately after mixing. In
mixed growing cultures, no mating pairs are found until the end of the exponential
growth phase, 6 or 7 days after inoculation. The observations drawn in Fig. 2 show
that the occurrence of reproductive differentiation is intimately correlated with
growth. Cells of opposite mating types were mixed in 10 ml. media in test tubes,
incubated under constant illumination. Then periodic observations were made with
the algal suspensions. If cells became sexually active, one could expect their auto-
matic copulation in the growing cultures. Cell-numbers were counted by using a
haemocytometer. Separately, in order to calculate the percentage of each cell stage
in the algal population, a small drop of the culture suspension was removed on a
slide-glass and fixed with aceto-carmine. Then the respective numbers of smaller,
larger and mated gametes were counted under the microscope. A mature zygote
was recognized as being resulted from two gamete cells.
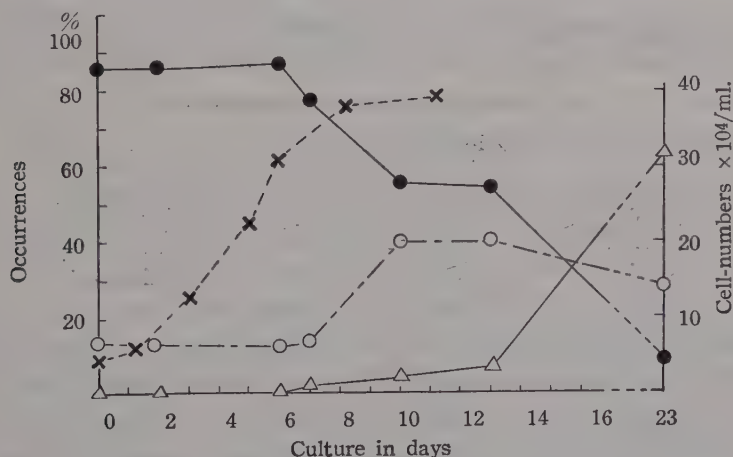
Fig. 2. Gametogenesis and copulation in a mixed growing culture: —●—; Larger
cells: —○—; Free smaller cells which are characteristic of being gametes: —△—;
Mated gametes: --×--; Cell-numbers in the culture.

Inasmuch as the appearance of sexuality seemed to be caused by the depletion
of something limiting to growth in the medium, further investigations were made
on this assumption.

When cells of opposite mating types are washed with distilled or tap water and
resuspended in water, they become fully gametic within 24 hours, and active copu-
lation can be observed shortly after they are mixed. The depletion of nitrogen
from the medium was found to result in abundant sexually active cells, and further,

light was found to promote this process. Table 1. shows the relation between abundance of gamete-formation and nitrogen concentration in the medium. Equal quantities of washed cells were transferred into media containing various concentrations of ammonium nitrate, and the suspensions were kept under constant illumination. After 24 hours occurrence percentage of gamete-formation was estimated by counting the respective numbers of smaller and larger cells in each medium. It is apparent that the more nitrogen in the medium, the less gametes are produced.

Table 1. Gamete-formation and Nitrogen Concentrations

NH_4NO_3 mg/l	Vegetative cells (larger) %	Gamete (smaller) %
400	86.0	14.0
40	79.4	20.6
4	23.9	76.1
0.4	2.3	97.7
0.04	6.1	93.9
0	3.5	96.5

In the absence of assimilable nitrogen, almost all cells began to divide into 8 smaller daughter swarmers all of which were gametic. On the other hand, in a medium containing assimilable nitrogen which allowed growth to continue, cells tended to divide to form only 4 cells, which on liberation showed no sexual activity. In both conditions division is presumably mitotic. Larger cells in the exponential phase of growth divided into smaller ones and were gametic, which could be confirmed by observing the gradual changes of the population within a mixed suspension in nitrogen depleted medium (Fig. 3). Furthermore it was noticed that 10–20% of the cells, though they were of the smaller size that was characteristic of being gametes, yet never showed any sexual phenomenon in the early periods (Fig. 2, 3).
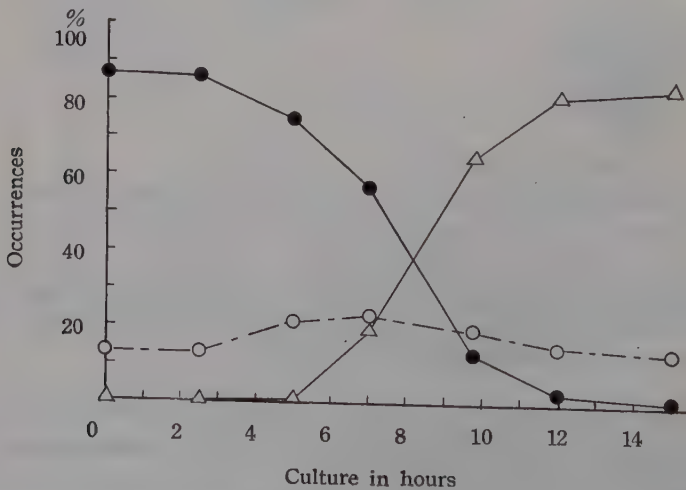
Fig. 3. Gradual changes of the algal population in a mixed suspension under a nitrogen depleted condition: —●—; Vegetative cells: —○—; Free gametes: —△—; Paired gametes.

Another experiment was designed to test the influence of nitrogenous medium

on the sexual activity of gametes. Cells of opposite mating types were incubated separately in a nitrogen-free medium for 15 hours under illumination, and equal volumes were then mixed and suspended in a dilute solution of KNO_3, NH_4Cl or KCl to a final concentration of 0.008 M. It had previously been observed that in 2.5 hours almost all sexually active gametes linked tightly by protoplasmic bridges and no more copulation could be expected. After the 2.5 hours the numbers of vegetative cells, free gamtes and paired gametes in each suspension were determined as before (Table 2).

It appears that not only gamete-formation but also copulation of gametes is inhibited in the presence of assimilable nitrogen.

The sexual activity of gametes kept in nitrogen-free media gradually decreases if they fail to copulate. However, a large number of mating

Table 2. Gametic Activity in Solutions of Salts

Salts (0.008M)	Vegetative Cells %	Free Gametes %	Paired Gametes %
KCl	13.8	36.1	50.1
KNO_3	11.5	73.4	15.1
NH_4Cl	14.2	69.6	16.2

pairs can readily be obtained by suspending cells of each mating type separately in a nitrogen-free medium, and illuminating them for 24 hours before they are mixed. In the continued absence of nitrogen, zygote become progressively paler in the course of time, and finally die. Fully matured zygote can only be obtained, if they are transferred into a nutrient medium under normal growing conditions.

Discussion and Conclusion

Studies on the life-cycle of *Chlamydomonas spp.* have already been made by many workers[2,3,13]. In most cases, a vegetative and a generative phase can be distinguished: this is true also for *Chl. sp. 24*. Though in many organisms reproductive differentiatiation can be induced by transferring the cells into distilled water or to the conditions of starvation as observed by Klebs and other investigators[1,4,12,14], the mechanism of such a change has not been clarified. As reported above, sexual activity could be induced in *Chl. sp. 24* by transferring cells into media deficient in nitrogen, and copulation of gametes was inhibited in the presence of assimilable nitrogen. But it remains yet to be shown whether or not the inhibitory effects of nitrogen on gamete-formation and on copulation of gametes are to be attributed to the same physiological mechanism.

Judging from the fact that sexuality appears under a certain condition of medium, the appearance of this character tells a need of some physiological conditions with the organism. Sager and Granick[15] reported in their studies on *Chl. reinhardi*, that the depletion or supply of nitrogen had an essential role in controlling the differentiation or dedifferentiation of sexual activity in the presence of assimilable nitrogen in its sexuality. In *Chl. sp. 24* as well, the dedifferentiation of sexual activity in the presence of assimilable nitrogen is most probable. Then it should

be attentioned that the loss of sexual activity could occur in a very short time without and noticeable enlargement or division of cells.

Accoording to Lewin, sexually active gametes of *Chl. moewusii* aud of *Chl. sp. 24* (personal communication) were readily obtained by flooding the cultures with distilled water, which had been grown on the surface of agar for 3–4 days and then kept in the dark for 24 hours. The present author has confirmed this observation in the latter species. Gametic activity was high in 15–30 minutes after flooding, but was lost when such a suspension was allowed to stand for several hours.

Thus it appears that sexual differentiation in *Chl. sp. 24* is closely bound up with growth, and a method has been evolved for obtaining active gamete suspensions. It remains to be shown whether in this species sexual behavior is controlled by a hormonal mechanism as has already been described in *Chl. eugametos* by Moewus[11].

The writer is deeply indebted to Dr. R. A. Lewin of the Maritime Regional Laboratory, Halifax, N. S., Canada, for much advice and suggestions and to Dr. H. Hirose of Kobe University for valuable council from his wide experiences in phycology.

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On the Standing Crop and Productive Structure of Phytoplankton Community in Some Lakes of Central Japan

by Shun-ei ICHIMURA*

市 村 俊 英: 湖沼プランクトンの現存量および生産構造

As a first step to sudy the plant community socio-ecologically, it is necessary to determine the production of matter, therefore analytically the standing crop and the productive structure of the community (Boysen-Jensen 1932, Monsi 1954). From this point of view some works have recently been done by Monsi and Saeki (1953), and Hogetsu, Ichimura et al, (1954) on the land plant community. On the phytoplankton community, also Hogetsu and Ichimura (1954), Ichimura (1954) have worked in similar direction. Although in addition to these works we have many descriptive works on the ecology of water plant, yet there remain many problems on the water plant community that should be fundamentally investigated and solved. In the present paper, the author deals mainly with the results of the survey in some lakes of central Japan, and he discusses the dry matter production and the productive structure of phytoplankton community.

Method

The quantity of phytoplankton has usually been determined by the cell number. However, the cell number alone does not give the quantitative indication of the standing crop or of the photosynthetic activity of phytoplankton. In order to estimate not only the quantity but also productive capacity of phytoplankton, determination of the chlorophyll content of water has received attention of some investigators in recent years, and it was pointed out by Wohlschlag and Hasier (1951) that the determination of chlorophyll content is very excellent as a semi-quantitative method of the measurement of the quantity of phytoplankton and as the best technique for the direct separation of phytoplankton from seston. So in the present research, this method was applied to water which was usually sampled serially with an interval of 1 or 2 metres from surface to bottom of the lake. The amount of water varied from 5 l to 10 l for the eutrophic lake and mesotrophic one, and 20 l for the oligotrophic and destrophic. The water was filtrated through a sheet of filter paper

* Botanical Institute, Faculty of Science, Tokyo University of Education.
東京教育大学理学部植物学教室

(Tōyō. No. 101, diameter 11 cm) with a suction apparatus and then the paper was washed with boiling water as Wohschlag and Hasler have done. The amount of chlorophyll, after the conversion of extracted chlor ophyll into pheophytin (Hogetsu and Ichimura 1954, p 285), was determined colorimetically. Photosynthesis and respiration of the sample water were determined by Winkler's method as mentioned in the previous paper (1. c). For the measurement of the light intensity in the depth of water the auther used a Madzuda photometer with special equipment.
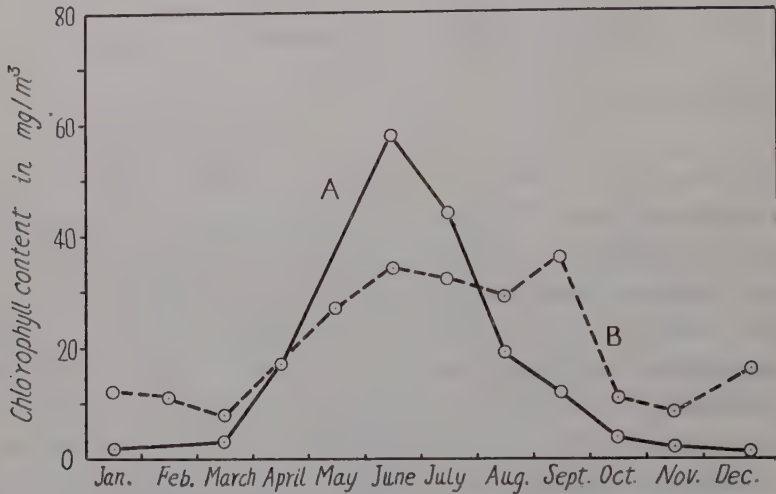
Fig. 1. Annual rhythm of chlorophyll content in lake water. A: Lake Nakanuma 1950-1951　　B: Lake Suwa 1949-1950

Lake types and chlorophyll content

It has already been observed by many investigators that phytoplankton shows a considerable variation in quality and quantity throughout the year and the course of the seasonal change varies with each lake.

Fig. 1 shows the annual rhythm of chlorophyll content of water in Lake Nakanuma* and Lake Suwa. Chlorophyll content is expressed as the mean value of the determination at various depths in the epilimnion. Chlorophyll content began to increase in both lakes in spring, and reached a maximum value early in summer, though the peak is acute in the former lake and the chlorophyll content decreased rapidly in the following season. In Lake Suwa the summer maximum continued for four months. The slight increase of plankton is September was brought with the propagation of diatom. Minimum value was observed in both the lakes in winter, but in Lake Suwa the value was osmewhat higher than at Lake Nakanuma. Dominants of phytoplankton were *Synedra, Diatoma, Eudorina* in Lake Nakanuma and *Melosira, Asterionella, Fragilaria, Anabaena* in Lake Suwa.

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* Nakanuma, located in south Ibaraki, has an area of 0.018 km², and a maximum depth of 13.4m and mean depth of 6.7 m.

The difference in the amount of phytoplankton or in the seasonal change of chlorophyll content between these lakes may be caused by the geographical difference of the environmental factors, as analysed in the previous paper (Ichimura 1954).

In order to assess the variation of chlorophyll content in lakes which belong to different lake type (Yoshimura. S. 1937), the author undertook to measure the chlorophyll content of some lakes. There were marked differences due to the lake type, as in Table 1 summarized.

As mentioned above, high content generally appeared early in summer or in autumn, and they varied roughly from 50 mg/m³ to 1 mg/m³ in eutrophic and mesotrophic lakes. In dystrophic and oligotrophic lakes, chlorophyll was commonly very scanty. It should, therefore, be noted that the lake-type may also be classified on the basis of chlorophyll content of lake water. Already the transparency has usually been used as an empirical indicator—

Fig. 2. Relationship between the transparency and chlorophyll content in lake water.

rather qualitative—of the productivity of lakes, however, it is necessary to make clear the quantitative relationship between the transparency and the amount of phytoplankton, for the purpose of indicating the productivity with the transparency exactly. Clark (1946, p. 331) pointed out in his work that phytoplankton itself may become sufficiently abundant to cause a significant decrease in transparency, and confirmed the influence of phytoplankton abundance on the transparency at George Bank. A definite correlation was found out between the transparency and the concentration of phytoplankton as measured by plant pigment. Atkins et al. (1954) found that there is an inverse relation between the transparency and the amount of phytoplankton, which was determined by spectrophotometric analysis of chlorophyll extracts, though the admixture of suspended inorganic matter may at time disturb the relation. However, this relationship has scarcely been examined in lakes. In the present papers the author obtained, in general, a curvilinear relationship between the transparency and the chlorophyll content in lakes, as in Fig. 2 indicated, except for the case of the organic matter in suspension abnormally abundant or when the lake received strong agitation by the wind or water circulation. Therefore, the average mass of phytoplankton can be estimated from the transparency. If the

facts above mentioned are practically applicable to many lakes, it may serve to clear up the meaning of the transparency as an indix of the productivity or as a criterion in the classification of lakes.

Vertical distribution of chlorophyll in lakes

The vertical distribution of chlorophyll also showed wide variation in every lake and in every season. The author, however, could obtain a practical evidence in support of the theoretical analysis which has been done on succession process of the phytoplankton community in previous paper (Hogetsu and Ichimura 1954). In vertical distribution of chlorophyll, there are two main types; i.e., the one is homogeneous distribution (Fig. 3. A–C) in which phytoplankton is distributed homogeneously, and the other is stratum distribution (Fig. 3. D–G) in which the stratification of chlorophyll concentration is observed and the most part of phytoplankton is found in the epilimnion, especially somewhere below the water surface. Both the types used to appear alternately in the course of year; the former generally appears during the circulation period and the latter clearly develops in the stagnation period. The distribution form of stagnated phytoplankton is very similar to the vertical distribution of the leaves in a plant community, which was made clear by Monsi und Saeki (1953).
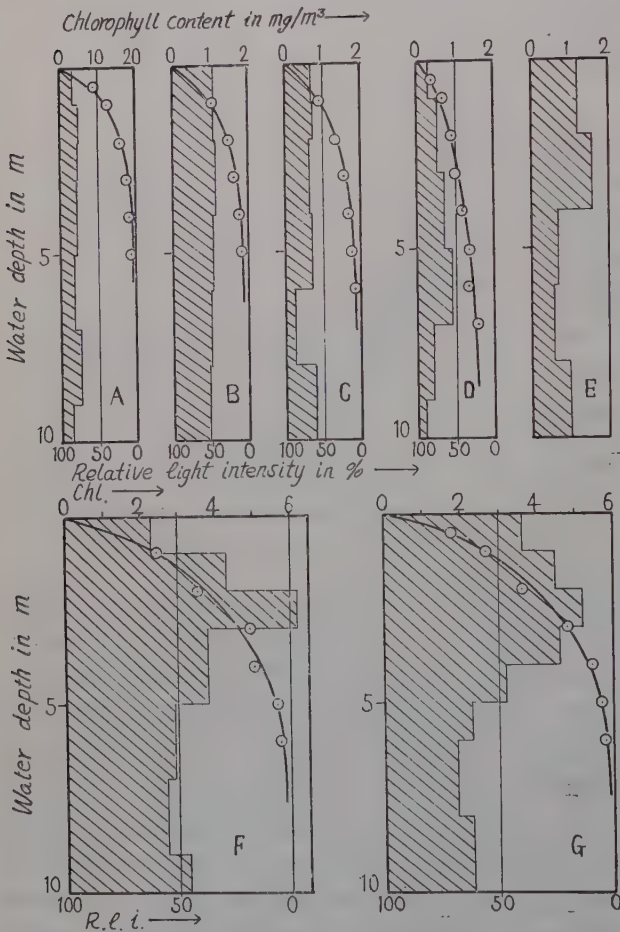
Fig. 3. Some examples of the vertical distribution of phtoplankton in lakes. A: Nakanuma, Oct. 15, 1950. B. Nakatsuna, July 14, 1953. C: Kizaki, July 24, 1953. D: Motosu, April 25, 1950. E: Nakatsuna June 16, 1950. F: Sōji, April 25, 1950. G: Yamanaka, Sept. 12, 1951.

One of the most important causes of the tranformation of the distribution form

or the productive structure may be the change of assimilation rate with the water depth. In Figs. 4 and 5 are illustrated assimilation rates of phytoplankton in a few lakes.

The rate of assimilation per unit mass of phytoplankton or the assimilation capacity of the water generally decreases with the increase of water depth. Their maximum occurs at the surface layer or somewhere below. Therefote, the dry matter production or the propagation of phytoplankton is vigorous at the upper layer of the community, so that as Talling (1955) also reported, the homogeneous distribution converted to the stratum one. The latter is, however, sometimes destroyed by circulation of the water due to wind or to density difference of water caused by temperature, and again returns to the former. At a shallow lake or pond in which water is easily subjected to greater agitation by wave or stream, phytoplankton generally distributes in the homogeneous type, but when water-bloom extremely develops, the exceptional stratum-type, which is similar to the productive structure of liane-community of the land plant (Monsi and Saeki, unpublished data), is usually

Fig. 4. Variation of assimilation rate due to water depth in lake. A: Suwa, December (1949) B: Nakamuna, September (1950) C: Nakamuna, October (1950) D: Suwa, September (1949)

formed (Fig. 6). Recently, Nothdurf (1954) had studied on the vertical distribution of phytoplankton in some shallow ponds and reported that the homogeneous distribution of chlorophyll was observed in many cases. In the summer or winter stagnation period, as an exceptional case, a zone which has the maximum chlorophyll content appears in the hypolimnion (see Fig. 5). Similar phenomena have been found by Manning and Judy (1941), Hogetsu and Ichimura (1954). This zone, however, is almost existing below the compensation depth so that the phytoplankton contained in this zone could not assimilate and naturally not propagate. Next experiment brought out these facts more clearly. The phytoplankton for experiment was taken from the various depth and the intensity of assimilation of each sample was measured with Winklers method under the same condition—under daylight and at 20°C. The chlorophyll content of each sample had been determined, too. The assimilation intensity was shown in O_2 mg per mg chlorophyll and a day. The phytoplankton found in the hypolimnion had no photosynthetic activity as illustrated in Fig. 7. Even though the phytoplankton in the hypolimnion had the photosynthetic activity, it could hardly perform photosynthesis (Fig. 5), because the light

intensity at the compensation depth measured by field works was only 3–5% of that of the surface and it was supported by some investigators that the compensation depth is about 2.2 times as large as the transparency depth. Hence the high concentration of chlorophyll found in the deep zone is doubtlessly caused by the sedimentation of phytoplankton which had propagated itself in the epilimnion.
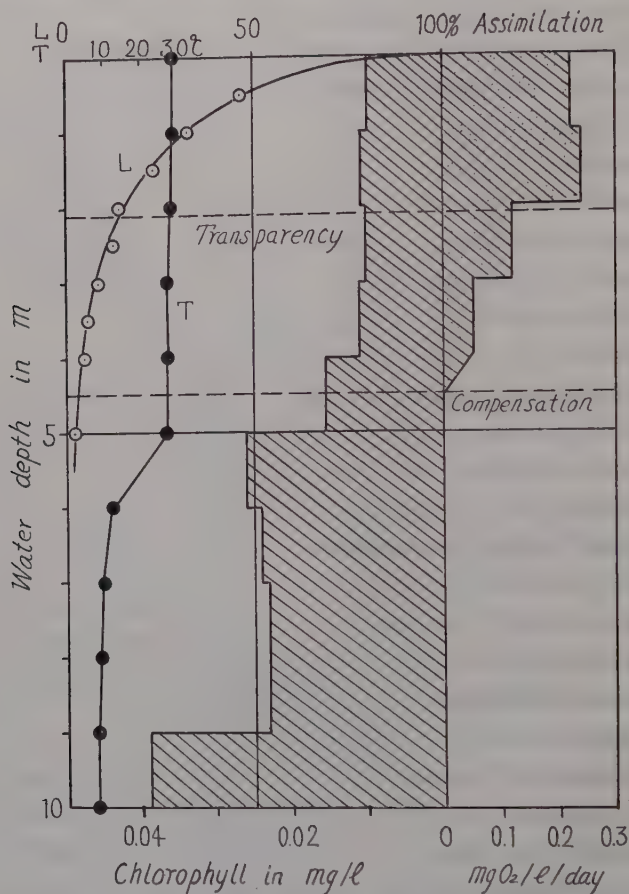
Fig. 5. Productive structure and productive capacity of phytoplankton community in lake. Nakanuma, Aug. 25. 1951. L: Relative light intensity. T: Temperature.

Determination of the amount of phytoplankton

In order to estimate the amount of phytoplankton from the chlorophyll content of lake water, we assumed the chlorophyll content of algae, regardless of species, in average 2.5% or 0.025 mg chlorophyll in mg dry substance, as in a previous paper (Hogetsu and Ichimura 1954), and this assumption was not so unreliable was proved by the measurement of photosynthesis (l. c.). In Table 3 the author indicated the results of monthly observation at Lake Nakanuma, i. e. the chlorophyll content, the phytoplankton estimated from it and the seston. The seston in summer is mainly consisted of phytoplankton, but in winter and early spring the non-chlorophyllic organisms or inorganic matters are main components of the seston.

Table. 1.　Average values of chlorophyll content in milligrams per cubic metre from surface to compensation depth in some lakes

Lake type	Lakes and waters	Maximum depth in m	Area km²	Date	Chlorophyll mg/m³	Dominants of phytoplankton
Eutrophic	Nakanuma	13.4	0.018	June 8, 1950	57.5	*Eudorina, Spirogyra*
				July 5, 1950	45.5	
	Sugao	1.7	3.8	Sept. 28, 1952	12.7	*Endorina, Melosira*
	Suwa	6.5	14.5	July 14, 1949	44.8	*Anabaena, Melosira*
				Aug. 11, 1950	35.3	*Synedra, Melosira*
	Tega	1.5	11.9	June 10, 1951	27.3	*Melosira, Fragilaria*
				July 20, 1952	22.6	*Melosira, Eudorina*
	Ushiku	1.5	4.5	April 11, 1952	19.2	*Arthrodesms, Staurastrum*
				June 29, 1952	17.1	*Spondilosium, Xanthidium*
	Rice fields (ail. 6 m)			July 17, 1952	16.1	*Spirogyra, Mougeotia*
				Aug. 20, 1952	14.6	
	(260 m)			July 15, 1952	26.7	*Spirogyra*
				July 27, 1952	21.8	*Oscillatoria, Nitzchia*
	(1042 m)			July 27, 1952	10.6	*Navicula, Pinnularia*
				Aug. 19, 1952	12.7	*Gonatozygon, Navicula*
Mesotrophic	Kawaguchi	15.2	5.8	Nov. 25, 1949	2.81	*Asterionella, Melosira*
				April 28, 1950	2.45	
	Kizaki*	29	1.4	June 16, 1953	1.64	*Tabellaria, Cyclotella*
				July 24, 1953	1.09	*Asterionella, Melosira*
	Nakatsuna*	12	0.14	June 16, 1953	1.12	*Melosira, Cyclotella*
				Aug. 4, 1953	2.10	
	Yamanaka	15	6.5	April 28, 1950	5.10	*Botoryococcus, Melosira*
				Sept. 12, 1951	4.21	*Melosira, Eudorina*
	Soji	16	0.87	Sept. 20, 1949	1.90	*Fragilaria, Synedra*
				April 10, 1950	3.92	
Oligotrophic and Dystrophic	Aoki*	62	1.9	July 26, 1953	0.35	*Melosira, Cyclotella*
				Aug. 20, 1953	0.36	
	Motosu	126	4.9	Nov. 27, 1949	0.10	*Asterionella, Botoryo-coccus*
				April 25, 1950	0.41	
	Sai	77	1.4	Nov. 28, 1949	0.80	*Asterionella, Melosira*
	Oze	9.5	1.7	Aug. 8, 1952	0.19(?)	*Melosira, Cyclotella*
	Tadenoumi			July 4, 1950	0.16	
	Oze-Bog lake	1-2	0.03- 0.05	July 10. 1451	0.5-1.0	*Ulothrix*
				Aug. 14, 1952	0.1-0.5	*Melosira*

* Data were measured by Saijo, Y. and Oshima, H. (unpublished)

Table 2, Seasonal change of chlorophyll content (mg/m³; upper figures). dry weight (mg/1) of seston (middle ones) and of phytoplankton calculated from chlorophyll (lower ones) in Lake Nakanuma, 1950-1951.

Depth in m	1950 June	July	Aug.	Sept.	Oct.	Nov.	Dec.	1951 Jan.	March	April
0	61.7	50.0	10.1	22.0	1.8	2.5	—	1.4	8.2	4.8
	3.35	2.30	0.45	—	0.85	1.01	1.46	1.75	3.58	1.37
	2.87	2.06	0.45	0.88	0.30	0.10	—	0.06	0.33	0.20
1	61.7	45.2	11.9	8.1	5.3	3.5	2.1	1.2	5.9	30.5
	2.75	4.00	0.60	0.70	0.45	1.20	1.21	1.07	3.08	2.62
	2.87	1.80	0.48	0.32	0.20	0.14	0.08	0.06	0.23	1.20
2	52.3	52.9	10.5	8.3	4.3	2.6	1.7	0.8	2.3	26.3
	4.30	2.97	1.75	0.65	1.50	1.39	1.60	2.11	4.22	2.10
	2.10	2.80	0.42	0.33	0.17	0.10	0.08	0.03	0.10	1.00
4	52.6	35.2	16.4	5.8	4.2	5.8	1.6	1.0	—	24.6
	3.05	1.90	0.89	0.42	0.90	1.43	1.70	1.45	2.86	2.30
	2.10	1.40	0.66	0.23	0.11	0.23	0.06	0.04	—	1.00
6	47.2	73.5	24.1	16.0	2.8	2.3	1.1	0.9	1.0	11.2
	3.35	—	1.00	—	0.85	1.15	1.80	1.30	—	2.95
	1.89	2.90	1.09	0.68	0.64	0.09	0.04	0.04	0.04	0.45
8	63.9	33.7	23.1	10.4	5.5	1.4	2.2	0.9	1.0	5.6
	3.10	1.85	—	0.78	0.85	1.34	1.15	—	2.56	2.56
	2.35	1.30	0.93	0.42	0.42	0.06	0.09	0.04	0.04	0.22
10	72.9	30.9	38.9	15.1	2.5	0.9	1.0	0.5	—	6.1
	3.90	2.15	—	1.23	1.85	2.45	1.70	1.10	—	3.60
	2.90	1.24	1.56	0.60	0.06	0.04	0.04	0.02	—	0.24
mean	58.7	45.6	19.3	12.2	3.8	2.7	1.6	1.0	4.0	15.6
	3.40	2.53	0.94	0.75	1.03	1.42	1.51	1.45	3.26	2.56
	2.47	1.72	0.67	0.46	0.23	0.11	0.05	0.04	0.15	2.50
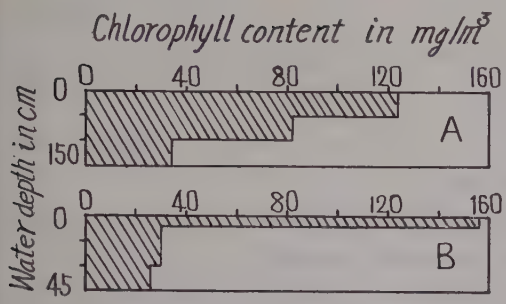
Fig. 6. Vertical distribution of phytoplankton in pool with water bloom. A: consisted with *Melosira italica, Eudorina elegance* (May 12, 1950) B: consisted with *Euglena sp., Chlamydomonas sp.* (April 24, 1951)
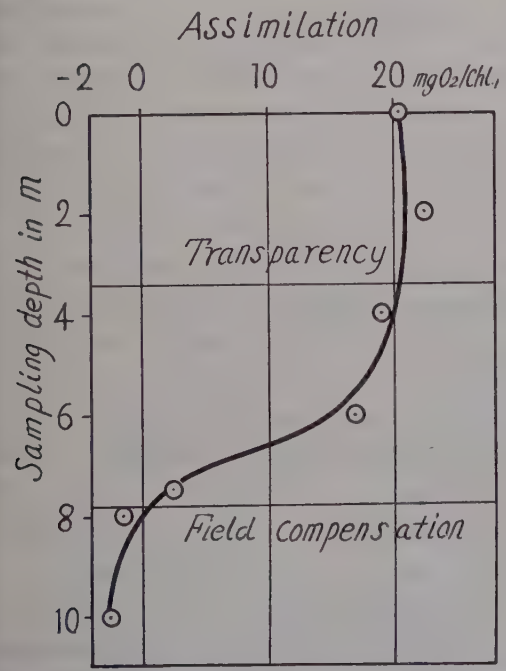
Fig. 7. Assimilation rate of phytoplankton(Lake Yamanaka, September 12, 1951). Sample water was collected from the various depth and the assimilation rate was measured under the same condition. The concentration of phytoplankton was determined with chlorophyll amount.

These seasonal changes accorded with those of the chemical composition of deposits (Saijō, Ichimura et al. 1954). In Lake Suwa, we also found a similar seasonal change in the composition of seston (Hogetsu, Ichimura et al. 1952).

Summary

The standing crop and productive structure of phytoplankton communities in some lakes of central Japan have mainly been investigated by chlorophyll extraction method.

1. Remarkable difference of chlorophyll content with lake type was ascertained. In summer, average values of the chlorophyll content in epilimnion are in maximum ca. 50 mg/m³ in eutrophic lakes, and less than 1 mg/m³ in oligotrophic and dystrophic ones.

2. The chlorophyll content in lakes varied not only in quantity but also in the vertical distribution,–the productive structure of phytoplankton community——, of which two main types, i. e., homogeneous distribution and stratum one (Fig. 3), were recognized and both the types appeared alter nately in the course of year, The process of the transformation of the two types was analysed on the basis of metabolism of phytoplankton.

3. The standing crop of phytoplankton was investigated with the amount of chlorophyll. It showed that in summer the seston was mainly composed of phytoplankton but in winter mainly inorganic matter and non-chlorophyllic organisms.

The author wishes to express his thanks to Prof. M. Monsi and Prof. K. Hogetsu,

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Effect of Auxin upon the Water Uptake of *Avena* Coleoptile

by Shizuko KOBAYASHI, Isao HATAKEYAMA and Joji ASHIDA*

小林静子・畠山伊佐男・芦田譲治：　燕麦幼葉鞘におけるオーキシンと吸水

When the plant cell elongates, it necessarily absorbs water. And the water absorption of a cell is conditioned either by (1) a decrease in the wall pressure or (2) an increase in suction power due to cell contents, or both. The second category involves 2a) an increase in the osmotic value of cell contents and (2b) an increase in the power of active water absorption.

Effects of auxin on the metabolism of tissues have been reported by many authors (e.g. see 1). For the understanding of action of auxin, however, it is important to determine how the water uptake is caused as a result of auxin-induced

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* Botanical Department, Faculty of Science, Kyoto University.

metabolic sequences, ——that is, to determine which of the above-mentioned alter-native mechanisms really operates in the final step.   Brauner *et al.*[3] have shown that the plastic extensibility of cell wall is increased by auxin under aerobic condi-tion, and Burström[4] proposed that the root growth of wheat is due to increased wall extensibility.   On the other hand, Thimann[10] ascribed the auxin action to the stimulation of active water absorption.

If the water absorbing power (whether osmotic or active) of cells were increased by the action of auxin, even the cells which have been in the state of incipient plasmolysis should absorb water when they are treated with auxin.   On the contrary, if auxin had an effect *only* on the wall extensibility, the water uptake would not occur, because this has not been limited by the wall pressure in the state of incipient plasmolysis.   In order to throw light on this critical point, the experiments here reported have been made.

Material and Method

The material was *Avena sativa*, Victory No. 1.   In order to avoid complication by microbes, sterile seedlings were used.   Seeds were soaked in 70% alcohol for ten minutes, and then in 0.01% solution of mercuric chloride for 25 minutes, after being rinsed twice in sterile water for 1.5 hr. each.   They were planted on Knop-agar which was supplemented with 2% of sucrose to reveal microbial contamination.

Coleoptiles, 26 to 28 mm in length, were decapitated 1 mm, and three 3 mm segments were cut from the upper part of each coleoptile, using a multiple-razor-blade cutter.   It had been found by preliminary experiments that these apical segments grew in aerated auxin solutions better than more basal ones, and did not signifi-cantly differ among themselves.   The segments were mixed together and twenty of them made a lot for each treatment.   The auxin concentration chiefly used was 1.0 mg/1 which proved to be most effective for the fresh weight increase of the coleoptile segments in 24 hours at 26°C.

O_2 uptake was measured by the direct method using Warburg's constant volume respirometer[11].   Thirty coleoptile section pieces (about 50 mg. in fresh weight) were suspended in the main chamber which contained 3 ml of distilled water or IAA solution.   Central well contained 0.2 ml of 10% potassium hydroxide.   The bath temperature was 26°C.   The rate of shaking was 60 strokes per minute.   Since buffer solutions decreased the sensitivity of coleoptile to IAA, they were not used as the basic medium for the experiments in which water absorption and O_2 uptake were measured.

Results

A. *Effect of auxin on the water uptake in hypertonic solution.*

After weighing, coleoptile section pieces were soaked in lactose solutions of

various concentrations. And their fresh weight was determined after 4 hours. There was no change in the weight with 0.14 molar solution. Hence this concentration was equivalent to the suction pressure of the cells. The value corresponding to this was 0.15 molar, when similar measurements were made with mannitol solutions.

It had been expected that the decrease in fresh weight of tissue pieces would cease when the sugar concentration surpassed the value of incipient plasmolysis. But the weight decreased even in 0.5 molar and higher solutions. Since the osmotic value at incipient plasmolysis could not thus be determined by weighing, the sugar concentration at which 50% of the cells were plasmolysed was determined. The value was 0.25 molar with lactose and 0.3 molar with mannitol.

Fresh weight of coleoptile sections, floated in 1 mg/1 solution of IAA with aeration, was measured every 4 hours. The water absorption progressed almost linearly within the first 8 hours of soaking. Therefore, a 4-hour treatment was used in the following experiment.
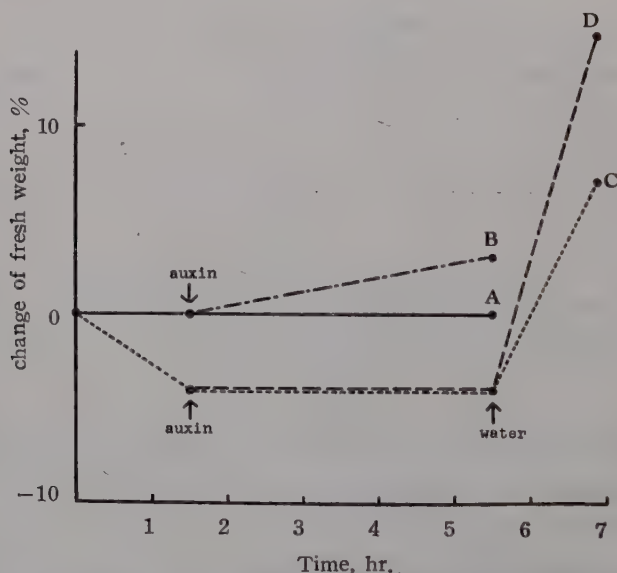
Fig. 1. Effect of auxin on the fresh weight of coleoptile sections immersed in lactose solutions equivalent to the suction force of cells (0.14 M) and to the osmotic value at incipient plasmolysis (0.25 M). A: 0.14 M lactose; B: O–1$^1/_2$ h. in 0.14 M lactose and 1$^1/_2$ –5$^1/_2$ h. in 1 mg/1 IAA+0.14 M lactose; C: 0–5$^1/_2$ h. in 0.25 M lactose and 5$^1/_2$–6 h. in water; D: 0–1$^1/_2$ h. in 0.25 M lactose, 1$^1/_2$–5$^1/_2$ h. in 1 mg/1 IAA+0.25 M lactose, and 5$^1/_2$–6 h. in water.

Coleoptile sections were divided into 4 lots. After weighing, two of them were soaked in 0.14 molar solution of lactose, and the other two in 0.25 molar. After 1$^1/_2$ hours they were weighed, and one lot from each sugar concentration was transferred to the solution of 1 mg/1 of IAA dissolved together with sugar at the respective concentration. The remaining lots were returned respectively to the sugar solutions of the original concentrations. And the weight was measured after 4 hours. The soakings were aerated throughout at 26°C. As shown in Fig. 1, the lot of

sections soaked in the 0.14 molar lactose solution which contained auxin, increased its weight, but that soaked in the 0.25 molar solution supplemented with auxin did not change its weight, just as not in the solutions without auxin. Results quite similar to this were obtained also when 0.16 and 0.3 molar solutions of mannitol were used.

It was revealed by these experiments that the water uptake was induced by auxin when the wall pressure was limiting it, but not when the cells were in the state of incipient plasmolysis. If the osmotic pressure of cell content or the active water-absorbing power were to be increased by auxin, the cells in the state of incipient plasmolysis should absorb water when acted upon by auxin. It may be suggested, therefore, that a decrease in the wall pressure is the main factor of the stimulation of water absorption by auxin. This suggestion, however, would be false if the suction force of cells is not increased by auxin in the dehydrated state as of the incipient plasmolysis, even though it might be in the normal osmotic condition. Hence respiration, which may serve as an indicator of cellular activity, was measured in the turgescent and the incipient-plasmolytic conditions.

B. *Effect of auxin on respiration in hypertonic solution.*

Active water absorption depends on cell respiration and this in turn is influenced by auxin [6,7,8 and 9].   If respiration of cells in the state of incipient plasmolysis
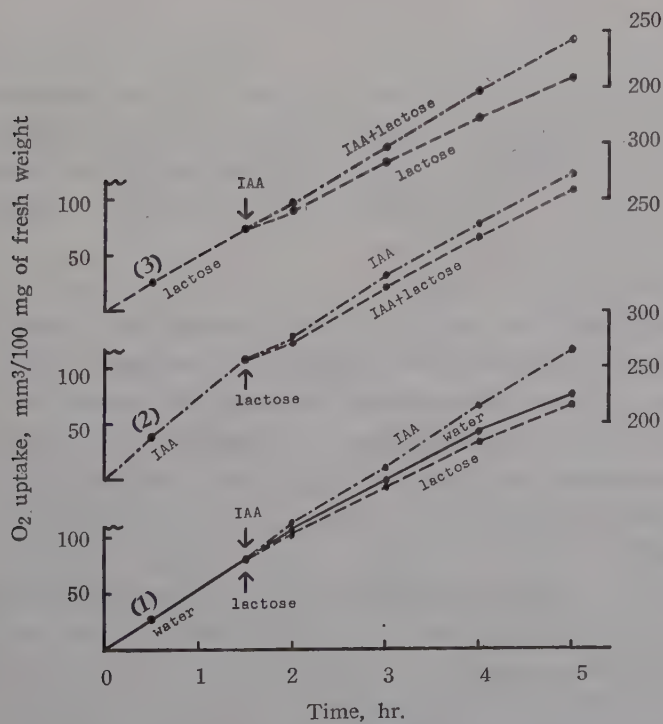
Fig. 2. O_2-uptake by coleoptile sections. Notations of the suspending medium: Water: distilled water; IAA: 1 mg/1 IAA solution; lactose: 0.25 M lactose solution,

failed to be stimulated by auxin, the active water absorption by such cells would not be promoted by auxin.

The O_2 uptake of coleoptile section pieces was measured for $1^1/_2$ hours in (1) distilled water, (2) 1 mg/1 IAA solution, and (3) 0.25 molar lactose solution. After this, the three media were supplemented from the side chamber with (1) IAA or lactose, (2) lactose and (3) IAA, respectively, and the respiration measurement was continued for additional $3^1/_2$ hours. Representative results are illustrated in Fig. 2, and summarized in Table 1.

Table 1. Abstract from Fig. 2

First medium ($1^1/_2$ h.)	Second medium ($3^1/_2$ h.)	Relative O_2 uptake in the second medium
(1) Water	{Water IAA Lactose	100* 124.2 95.1
(2) IAA	IAA	120.4
(3) Lactose	Lactose	92.5
(2) IAA	{IAA IAA+lactose	100* 91.5
(3) Lactose	{Lactose IAA+lactose	100* 123.5

    * as standard.

Lactose suppressed respiration in water, as well as in the auxin solution which stimulated respiration. However, it was also certain that auxin increased respiration even in the lactose solution which caused incipient plasmolysis. The experiment (3) in Fig. 2 and Table 1 corresponded to C and D in Fig. 1. It was thus revealed that, in the state of incipient plasmolysis, the water absorption did not occur even though the respiration was stimulated by auxin.

Quite the same results occurred from the experiments using mannitol.

It is highly probable that the active water uptake will not increase if respiration does not increase. But the reverse can not necessarily be expected. It is impossible to say from the above experiments that the water absorbing power of coleoptile cells must be increased by auxin even when they are in the state of incipient plasmolysis. The results, however, have deprived a positive proof of the following plausible objection:

The fact that the auxin does not stimulate the water absorption of cells at incipient plasmolysis is to be ascribed to such a general lowering of cell activity as to be non-responsive to auxin action.

Discussion

Thimann [10] added mannitol to an auxin solution to reduce the elongation of

stem pea stem osmotically.   The rate of elongation was not found to increase when the
stem piece was transferred from this sugar-auxin solution to an auxin solution of low
osmotic value.   So he inferred that the extensibility of cell wall was not " plasticized "
by auxin.   However, the present investigation has shown that, when *Avena* coleoptile
pieces are transferred from an osmotic medium to water, those which have been
acted upon by auxin absorb more water than those which have not been.   Thimann's
experiment differs from the present one as follows ;

	Material	Auxin	Mannitol conc.	Inhibition	Quantity measured
Thimann's exp.	Pea	NAA	0.08 M	ca. 50%	Length
Present exp.	Oat	IAA	0.3 M	100%	Wet weight

And it may also be worthy of note that, in the present experiment, the material
had been in the auxin solution of high tonicity for 4 hours when it was transferred
to the solution of low tonicity, while the treatment in the first solution lasted for
8 hours in Thimann's case.   It may not be good enough to transfer the material
into the good-to-grow condition when little time is left for the growth at the
maximal rate.

The fact that the water absorption is increased by auxin in the sugar solutions
equivalent to the suction tension of cells cannot be explained by assuming that the
au in action rests upon the effect on water permeability, but it may be accounted
for by either of the two alternatives: a) auxin reduces the wall pressure or b)
auxin increases the osmotic or active suction force of the cell content.   But since
auxin does not increase net water uptake at the incipient plasmolysis, the second
alternative is a weaker hypothesis, and the former, " plasticizing " of cell wall, is
left undenied.

Bonner *et al.* observed that, when the tissue slice of topinamber, having an
osmotic value of 0.12 mol, was affected by auxin, it took up water from 0.4 molar
mannitol solution which was to plasmolyse unaffected cells.   And he concluded that
the active water absorption must be induced by auxin.

However, Burström[5] cited the results obtained by Schulbach and Knoop, and
by Wiklund that the osmotic value of topinamber tuber was much higher than 0.12
mol, and he suspected that the method used by Bonner *et al.* was not adequate
enough.   Burström[5], measuring the osmotic value of the topinamber tuber and
conducting similar experiments as Bonner *et al.*, concluded that the stimulation of
active water absorption by auxin was not inferable.   Brauner and Hasman[3],
using potato tuber tissue, have arrived at a conclusion that auxin increases exten-
sibility and plasticity of the cell wall under aerobic conditions.   Burström[4] holds
a view, especially with respect to the growth of wheat root, that the growth in
isotonic and hypotonic solutions is different from that in hypertonic solutions in the

underlying mechanism pertaining to the cell wall. He has denied the role of active water absorption in the cell growth.

An objection is possible that the metabolic activity may not be stimulated by auxin in the state of incipient plasmolysis, even though it might be under normal osmotic conditions. A positive support of this objection was removed by respiration measurements. A possibility should be reserved, however, that stimulation of active water absorption by auxin may be inhibited at incipient plasmolysis, even though the respiration can really be stimulated under this condition.

Another possibility is that the osmotic value of cell content cannot be increased by auxin in the state of incipient plasmolysis, even though it can be when cells are in the normal osmotic condition. An increase in the osmotic value due to auxin is not very probable with etiolated *Avena* coleoptile which is soaked in the medium containing no mineral salts.

When the coleoptile pieces which had been kept at the incipient plasmolysis were transferred to water, those affected by auxin (D in Fig. 1) increased their weight much more than those unaffected (C in the same figure). This strongly suggests that the cell walls had been " plasticized " by auxin, although there could be some residual growth effect of auxin.

Summary

The fresh weight of sectioned pieces of *Avena* coleoptile did not increase in an auxin solution if this contained lactose or mannitol to cause incipient plasmolysis of the cells. When transferred from the sugar solutions to water, the pieces treated by auxin absorbed more water than those without auxin action. The results suggest that auxin acts to decrease the wall pressure, rather than to increase the absorbing power of cell contents.

O_2 uptake, as well as its stimulation by auxin, was not much inhibited in the state of incipient plasmolysis. This partly answers the objection that metabolic activities which support active water absorption might be inhibited under the abnomal osmotic condition.

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Transmission Rate of the Photoperiodic Stimulus Across the Graft Union in *Pharbitis Nil* Chois

by Shun-ichiro Imamura* and Atsushi Takimoto*

今 村 駿 一 郎・滝 本　敦：　アサガオの接木における日長刺激の移動速度

The floral stimulus can readily be transmitted across the graft union in many
plants [1,2,3,7,8,9,10,11]. This is also the case in *Pharbitis Nil* [4]. Short day treatment
of one partner of the graft can induce flower initiation in the other noninduced
partner. In a previous paper the transmission rate of the floral stimulus from a
photoinduced branch to another non-induced one was reported in two-branched plants
of *Pharbitis* [6]. In the present paper investigations of the transmission and its rate
across the graft union are described.

Material and Methods

The material used was the same as in the previous paper [4,5,6]. When the
plants under continuous illumination attained the size desired, the cotyledons were
removed, the stems of two plants were shaved longitudinally to the cambium on a
1–1.5 cm stretch above the cotyledonary node and the two shaved surfaces were
bound together with a hemp string. After a union of the tissues was established,
about 10 days or more after the grafting, the string was removed to avoid eventual
effects of girdling. About 2 weeks after grafting, the main axes were removed
above the young just expanded leaves. Various modes of removal of leaves and
buds were adopted to find out their influence upon the transmission of the stimulus.

* Laboratory of Applied Botany, Faculty of Agriculture, Kyoto University.
　（京都大学農学部　応用植物学研究室）

Dark treatment of 16 hours' duration per day was given by enclosing the donor leaf in a bag of light-proof paper or by transferring the plants to a dark room. After five such treatments they were removed to a continuously illuminated bench, and examined for initiation of flower primordia at the end of the experiment.

The principle of measuring of the transmission rate was the same as described in the previous paper [6]. In 3 time-measuring lots, comparable plants were used, grafted in the same way as in the experimental lot. The main axes of both partners were decapitated above the nodes, leaving the donor leaves and the receptor buds intact and removing all other leaves and buds. In each lot the dark treatment was started a varying number of days after the removal of the main axes. The average position indicating number[*] of nodes bearing the first flower on the receptor bud was increasing with increasing delay of the start of the dark treatment after the topping. This relation could be used as a measure of time needed by the stimulus to reach the receptor bud.

Transmission of the floral stimulus across the graft union. The experiment consisted of 6 lots. The first and second lots of 20 grafts each had a donor leaf and a receptor bud, and all other leaves and buds were removed. Dark treatment was given to the first lot by placing it in a dark room and to the second by enclosing the donor leaf in a bag. In the third lot the axillary bud of the donor leaf was also left intact and dark treatment was given in a dark room. The fourth lot had the leaves and their axillary buds of both donor and receptor branches intact. Dark treatment was given by means of a bag, so that the leaf on the receptor plant remained under continuous illumination throughout the experiment. Two other lots of 12 grafts each served as controls. In one control lot both partners of the graft were entirely defoliated and debudded except for two uppermost buds and subjected to dark treatment. In another control lot both partners had the uppermost leaves and their axillary buds intact and received no dark treatment.

The results are given in Table 1. All the plants of the first lot, which had only the donor leaf and the receptor bud intact and were treated in the dark room, initiated flower primordia and 6 plants out of 20 had terminal flowers. The first flower primordia appeared on the 2.6th node on the average. The behavior of the second lot in which the dark treatment was given by enclosing the donor leaf was almost the same as that of the first lot. This indicates that the induction occurs only in leaves and the illumination of the stem has no influence upon the photoperiodic response. In the third lot, in which the axillary bud of the donor leaf had been allowed to develop, the reaction in the axillary shoot of the donor leaf was far stronger than that in the shoot of the receptor plant. The former initiated 19 terminal flower primordia in contrast to 6 of the latter. Moreover, one plant out of 23 receptors had no flower buds. The position of the first flower was lower in

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*　i.e. position of the nodes in their succession on the stem: 1st, 2nd. 3rd ⋯⋯⋯ and so on.

the donor than in the receptor, indicating that the stimulus reached to the bud of the receptor later than to that of the donor.

In the fourth lot where the uppermost leaves and their axillary buds of both donor and receptor branch were left intact, the difference in the reaction of the two axillary shoots was more significant. All the axillary buds of the donors developed flower primordia and 19 out of 20 had terminal flowers. 8 shoots of the receptors did not initiate any flower primordia and none of them formed a terminal flower. The positions of the first flowers on both partners were also significantly different. All the defoliated control plants treated in the dark room and the control plants kept under continuous illumination did not develop flower primordia and remained strictly vegetative.

From these data it may be concluded that the stimulus can reach to the receptor bud more readily with enough intensity to cause flower initiation if the donor leaf is deprived of its axillary bud and the receptor bud of its subtending leaf.

Transmission rate of the photoperiodic stimulus across the graft union. As time-measuring plants both partners of the grafts were used. The axis was removed above the second node. The second leaf and its axillary bud were left intact and all other leaves and buds were removed. The plants received short day treatment 1 day, 2 days and 3 days after the removal of the main axis. Control plants which received no dark treatment initiated no flower primordia.

In one experimental lot 40 grafts were used. The second leaf of one partner was used as donor leaf and the third axillary bud of the other partner as receptor bud. All other leaves and buds were removed. Then the donor leaf was enclosed in a bag. In the course of experiment 4 plants were discarded on account of damaged petioles. After 14 days it was revealed that the first flower primordia appeared on the 3.08th node on the average, as shown in Table 2a. In the time-measuring lot which received the dark treatment 1 day after the topping, the flower primordia appeared on the 2.16th node, i.e. on lower nodes than in the experimental plants. In the plants treated one day later, i.e. 2 days after the topping, the first flower primordia were initiated on higher nodes, namely on the 3.37th node (Table 2b).

The lag in the arrival of the stimulus at the receptor bud was calculated by interpolation to be 42.2 hours, which is due to the difference in the path length. As shown in Table 2c, the latter was 117.4 mm; we can calculate that the stimulus moved with the rate of 2.8 mm per hour on the average.

In another group, the subtending leaf of the receptor bud was left intact in order to find out whether the leaf of the receptor subjected to non-inductive photoperiod may have any influence upon the transmission rate. 2 plants out of 28 were not induced to initiating flower primordia. The first flower appeared on the 2.75th node on the average in 26 plants, i.e. in somewhat lower position than in the former experimental group, but the path length of transmission was far shorter. On calculation the transmission rate was 2.4 mm per hour.

The results of another experiment carried out at an earlier date than the above mentioned one are represented in Table 3. In 3 time-measuring lots the plants received dark treatment immediately, 1 and 2 days after the removal of the main axis, and the average position indicating numbers of the first flower were 2.00, 2.33 and 3.15, respectively. The experimental plants initiated the first flower on the 3.86th node on the average. This shows that in the experimental plants the stimulus did not reach to its receptor bud within 2 days. The difference in the path length of the stimulus was 127.2 mm. From these data we can only say that the stimulus traveled at a rate of less than 127.2/48, i.e. 2.7 mm per hour.

Discussion

From the above experiments, the values of the transmission rate across the graft union were found to be 2.8 or 2.4 mm per hour. As reported in the previous paper, in plants with two branches the transmission rates were found to be 3.8, 2.7, 2.9 and 2.6 mm per hour [6]. Comparing these values we may conclude that there is no significant difference in the transmission rate between grafted and two-branched plants. The graft union is of no significant hindrance in the transmission of the floral stimulus in *Pharbitis Nil*. But we must warn against the generalization of this conclusion to other plants, because the transmission of the stimulus in this plant seems to occur with remarkable facility as compared with other short day plants.

The leaf on the receptor which received non-inductive photoperiod seems also to have no serious effect upon the transmission rate. This is not quite in agreement with the results obtained in the 4th lot of Table 1. There are indications that the subtending leaf on the receptor exposed to continuous light has markedly inhibited the response. But attention must be paid to the fact that 2 plants out of 30 did not initiate flower primordia and were not considered in the calculation.

In calculating the results, the assumption was made that the transmission rate is constant in every direction and in all organs concerned. But preliminary observations indicate that this is not strictly the case.

Comparing the results of the 4th and 5th lots in Table 1, where the reaction of the axillary bud of the donor leaf exceeds that of the receptor bud, it seems very probable that the stimulus decreases in intensity on its way. Concerning these points detailed experiments are going on, the results of which will be reported in near future.

Summary

The transmission rate of the photoperiodic stimulus across the graft union in *Pharbitis Nil* was found to be 2.8 and 2.4 mm per hour. The stimulus travels in the grafted plants approximately with the same velocity as in the two-branched plants; the graft union has no significantly hindering effect upon the transmission of the stimulus.

Table 1. Transmission of the floral stimulus in graft union. (Sown on 24th August, grafted on 7th September, experiment started on 21st September, 1949)

No. of lots	Treatment			Observed plants	No. of grafts	No. of plants with flower primordia	No. of plants with terminal flower primordia	No. of plants without flower primordia	Average position indicating number of the 1st flower primordium
	Axillary bud of the donor leaf	Subtending leaf of the receptor bud	Mode of application of dark treatment						
I	removed	removed	dark room	receptor	20	20	6	0	2.69±0.151
II	removed	removed	leaf enclosed	receptor	20	20	6	0	2.45±0.198
III	intact	removed	dark room	donor	23	23	19	0	1.87±0.130
				receptor	23	22	6	1	2.91±0.146*
IV	intact	intact	leaf enclosed	donor	20	20	19	0	1.60±0.112
				receptor	20	12	0	8	4.17±0.520*
V	leaves of both partners removed, buds intact, (defoliated control)		dark room	both plants	12	0	0	24	
VI	leaves and buds of both partners intact (light control)		no dark treatment	both plants	12	0	0	24	

* Plants without flower primordia are excluded from calculation.

Table 2a.　Position of the first flower primordium on indicator bud of receptor plant induced by the donor plant grafted to it.　(Sown on 10th, grafted on 22nd August, experiment started on 8th September 1951)

Group	Number of grafts	Path length of the stimulus in mm.			Total	Average posion indicating number of the 1st flower primordium
		Petiole of the donor	Stem of the donor	Stem of the receptor		
I*	36	77.5 ±1.95	56.4 ±3.27	50.8 ±3.25	184.9 ±5.77	3.08±0.115
II**	28	64.0 ±2.44	48.8 ±3.63	40.9 ∓4.50	153.7 ±6.74	2.75±0.216***

* Subtending leaf of the receptor bud removed.

** Subtending leaf of the receptor bud remained intact.

*** 2 Plants without flower primordia are excluded from calculation.

Table 2b.　Position of the first flower primordium on the axillary bud of donor leaf in rela tion to the delay of the dark treatment after removal of the main axis.　Time-measuring lots.

Time elapsed from the torping to the start of dark treatment in hours	24	48	72
Numbee of plants	73	75	24
Petiole length of the donor leaf in mm.	62.9±1.56	72.0±1.61	67.4±3.00
Average position indicating number of the 1st flower primordium	2.16±0.076	3.37±0.065	4.04±0.204

Table 2c.　Calculation of transmission rate

Group	Average path length in experimental plants in mm	Average path length in time-measuring plants in mm	Difference in path length in mm	Retardation of stimulus in hours	Transmission rate, mm. per hour
I	184.9	67.5	117.4	42.2	2.8
II	153.7	67.5	86.3	35.6	2.4

Table 3a.　Position of the first flower primordium on indicator bud of the receptor plant induced by the grafted donor plant. (Sown on 25th May, grafted on 22nd June, experiment started on 9th July, 1951)

Number of plants observed	Path length of the stimulus				Average position indicating number of the 1st flower primordium
	Petiole of the donor	Stem of the donor	Stem of the receptor	Total	
35	78.3±2.51	61.1±2.34	59.5±3.13	198.9±5.14	3.86±0.101

Table 3b. Position of the first flower primordium on the axillary bud of the donor leaf in relation to the delay of dark treatment after removal of the main axis.

Time-measuring lots

Time elapsed from the topping to the start of dark treatment in hours	0	24	48
Number of plants	39	30	26
Petiole length of the donor leaf	70.6±2.35	64.7±2.02	71.7±2.40
Average position indicating number of the 1st flower primordium	2.00±0.073	2.33±0.100	3.15±0.091

Table 3c. Calculation of transmission rate

Average path length of experimental plants in mm	Average path length of time-measuring plants in mm	Difference in path length in mm	Retardation of stimulus in hours	Transmission rate in mm per hour
198.9	71.7	127.2	>48	2.7>

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有絲分裂の生体観察に利用できる植物材料

沢 村 正 五*

Shogo SAWAMURA: Plant Materials Available for the Investigation on the Mitosis *in vivo*

植物組織における生体観察の実験材料として望ましい条件をあげると，(1) 四季を通じ材料の得られるもの，(2) 実験室的栽培管理の容易なもの，(3) 核分裂像の観察容易なもの，の三点である。*Tradescantia* の雄芯の毛の細胞は生体観察の材料としてすでに前世紀の終りから知られている唯一のものである。

最近 Bajer (1952) は *Hymenophyllum* の生体核分裂の観察及び *Haemanthus* その他の内胚乳における核分裂の生体観察に大いに成果をあげている (1953)。一方固定観察における 最適の実験材料である *Allium* 及び *Vicia* 属の材料において mitosis の生体観察が達せられるならば細胞学的に極めて有意義なこと�>解せられたので，筆者は *Allium fistulasum* (2*n*=16) 及び *Vicia faba* (2*n*=12) の mitosis の生体観察について実験に着手し，ある程度の成果を得た。

本文においてはこれらの材料による生体分裂細胞の観察経過についてその大要を述べる。

実 験 方 法

(1) *Allium fistulosum*

A. fistulosum の花蕾全体は一般に葱坊主といわれ，一つのうすい膜苞につ�>まれている。

この親指程度内の葱坊主をとりその膜質をとりのぞくと多数の花蕾がある。予め蒸溜水を一滴おとしたスライドグラス上になるべく小さい花蕾を花梗のついたま�>とりだしてのせる。解剖顕微鏡下にこのスライドグラスのま�>のせて外花蓋の基部よりや�>上部をメスで切断し，2 本の柄付針を操作して外花蓋をひらくと花蓋中の葯及び子房はきりはなされ，無傷の小形の内花蓋3片が得られる。

かくしてこの内花蓋の 1 片をカバーグラス上におき寒天薄板法 (和田1943) によつて寒天薄板に封じ，下向にして湿室中に入れ観察並に培養する。

(2) *Vicia faba*

V. faba の発芽後まもない稚苗の頂端につ�>まれている成長組織 (図A) を蒸溜水を一滴おとしたスライドグラス上にとりだし，解剖顕微鏡の下で組織の基部を切断した後，同じく 2 本の柄付針を操作して中から極めて若い托葉の小片をとりだし，場合によつては再びメスで他の組織より切りはなす。これをカバーグラス上にのせ寒天薄板法によつて封じ湿室中に入れて観察並に培養する。注意して操作すれば 3 片以上の托葉小片が得られる。

以上の操作に使用するメスは眼科用細身の鋭利なものが必要であり，柄付針も出来るだけ尖端の細くしたものを使用する。また従来植物材料の生体観察にはガラスリングによる湿室を使用しているが，顕微鏡のコンデンサーレンズが長焦点でない場合，高倍率の観察では屢々不便をきたすので筆者はセルロイド製スンプ台板を利用し，下に封蠟でカバーグラスを密着させて穴の底をふさぎ，一方寒天薄板によつてカバーグラス上に封じられた試料を下向にしてこの穴の中央にくるようカバーグラスの二方に少量のリセリンをつけて台板に固着させて観察している (図B)。

リング湿室に比較してこのプレパラートの取扱いは至極簡便である。但し長期に亘つて観察する場合には内部の寒天板が乾燥しないように工夫する要がある。

実 験 結 果

A. fistulosum の花蓋及び *V. faba* の托葉の細

* 宇都宮大学学芸学部生物学教室

胞において mitosis の観察のため調査した 1 %
寒天液に含まれている蔗糖濃度との関係は第1表
の通りである。

A. fistulosum では蔗糖の 8% 及び 9% 処理
によつて mitosis は前期のはじめより進行し完了
する。

殊に 9 % の濃度では実験第 2 日目にさらに
mitosis の発現が観察される。

V. faba においてはその mitosis の発現は蔗糖
濃度の 7% より 9% にわたつてみられるが, 7%
では托葉縁辺の大きな細胞における核分裂像では
染色体が往々膨潤して観察が困難となる。

8% 及び 9% が好適で特に 9% の蔗糖濃度で
は実験第 2 日目同じく mitosis の発現が行われる。

これら 2 種の材料において mitosis の発現が蔗
糖濃度のいずれも 9 % で行われる事は生理的に
細胞が要求する滲透圧が同程度であることを意味
するものと解する。

核分裂の前期より細胞板の両母細胞膜に到達するま
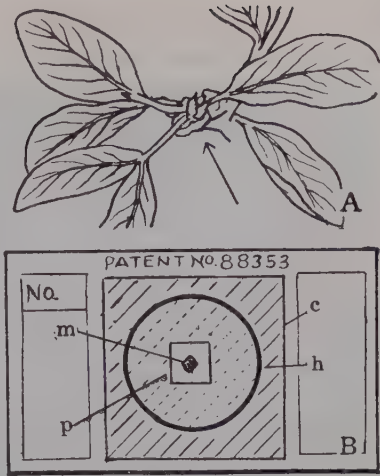
A. Vicia faba の稚苗における成長組織(矢印)。
　この中にある若い托葉を実験材料とする。
B. セルロイド製スンプ台板を利用した生体観
　察のためのプレパラート。
C, カバーグラス(24×24mm)。h, スンプ台
　板の穴(径約 18mm)。p, カバーグラス下面
　の寒天薄板 (約 5mm 平方)。m, 寒天板で
　封じられた材料。

でに要する時間はおよそ A. fistulo-
sum では 6 時間, V.faba では 4 時
間を要するが, 詳細な調査は今後の
実験によらなければならない。

V. faba において托葉周辺の分裂
細胞では終期, 隔膜形成体及び細胞
板の漸進的発達が分析的に観察され
ることは興味あることで, これにつ
いては別に報告した (沢村 1955)。
その際, 核の周囲をつゝむ細胞質の
活動とこれによつて成長する細胞質
紐の発達が明かに観察される。細胞
質紐の発達は紡錘体の形態的完成及
び隔膜形成体の母細胞膜への到達に
も重要な役割を果すものであつて図
10, 11, 16 はこれらの関係を示すも
のである。さらに紡錘体の独立性,
非混合性という和田 (1935) の見解
はこれら 2 種の材料の分裂細胞にお
いても観察される。

　また上述の方法に基ずいて V.
faba を材料として Caffeine 処理に
よる 2 核細胞及び MH 30 による染

第 1 表　1% 寒天液中に含まれる蔗糖濃度と核分裂との関係

実験材料　　蔗糖濃度	Allium fistulosum の内花蓋細胞	Vicia faba の托葉細胞
2 %	処理時, 後期にある分裂像暫時にして膨潤不明	分裂核を全く観察せず
4 %	処理時, 終期にある分裂像のみ進行完了	処理時, 終期にある分裂像のみ進行完了
5 %	処理時, 後期のはじめにある分裂像より進行完了	
6 %		処理時, 中期にある分裂像より進行完了。前期核は 逆行
7 %	処理時, 前期にある分裂核は逆行。その他の分裂像は進行完了	処理時, 前期にある分裂核より進行完了。但し紡錘体の膨潤する場合多し
8 %	処理後, 前期の核及び静止核より核分裂進行完了	
9 %	同上。さらに処理翌日, 核分裂発現し完了	
10%		処理時, 前期のはじめにある分裂核は往々進行抑圧される
12%		処理時, 前期の終り頃にある分裂核より進行完了
13%		処理時, 中期の終りにある分裂核より進行完了
14%		但し, 処理翌日細胞の半数は原形質分離
15%		処理直後, 静止核構造の変化顕著。仁観察, 終期分裂像のみ概ね進行完了。翌日, 全細胞原形質分離。

色体行動の異常を観察している（沢村 1956）。なお *V. faba* による生体分裂細胞の実験は冬季といえども簡単に材料が得られるばかりでなく，染色体数は少なくしかもその特徴ある染色体の形態が観察され（図14），将来 M 染色体の行動を生体細胞において追究することも至難なことでないと考える。以上 2 種の材料の組織細胞は寒天板に封じられたプレパラートのまゝ 20 日以上もその生活を持続し，原形質流動及び色素体の発達などが観察される。

考　察

Allium fistulosum 及び *Vicia faba* の 2 種の植物においてその mitosis を生体観察によつてたしかめることができたが，単に核分裂の観察のみならず生体分裂細胞に対する物理的あるいは化学的作用を調査する目的をもつて本研究に着手したものである。従来 *Vicia faba* の根端細胞を材料とした固定観察によつて物理化学的作用の研究が行われたが，今後生体観察によつても追究できることは実験細胞学的に重要な意義をもたらすものと思われる。

A. fistulosum の花蓋の分裂細胞の生体観察の結果は蔗糖濃度を考慮することによつて *Allium* 属の全般における mitosis の観察も可能であろう。実験材料として *A. fistulosum* の蕾は宇都宮においては 3 月下旬より 6 月下旬までの間に制約されるが，その他の *Allium* 属を使用すれば 8 月乃至 9 月初めまで実験を行うことができる。

長い間この種の実験材料として *Tradescantia* が主たるものであつたが，これら 2 種の材料が加わることは生体細胞の研究に一層の重要性を与えるものと考える。

終りに際して本研究に便宜をはからわれた本学奥野博士並びに種々有益な助言をいただいた東京大学教授和田博士の御厚意に深謝の意を表する。

要　約

Allium fistulosum の若い内花蓋細胞及び *Vicia faba* の若い托葉細胞における有絲分裂の生体観察の方法及びその経過を述べた。

これら 2 種の植物の細胞分裂は固定像として親しまれた材料であるが，今後生体観察を通じて Cell biology に貢献できるものと考える。

Summary

The studies on the mitosis *in vivo* were carried out on young petal cells in *Allium fistulosum* and stipular cells in *Vicia faba*, and the methods and the observational results are described in detail.

On the standpoint of cell biology it is very important that the mitosis in *Allium* and *Vicia*, the most common materials in fixed preparations, is now studied in the living state of the cell.

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Plate I
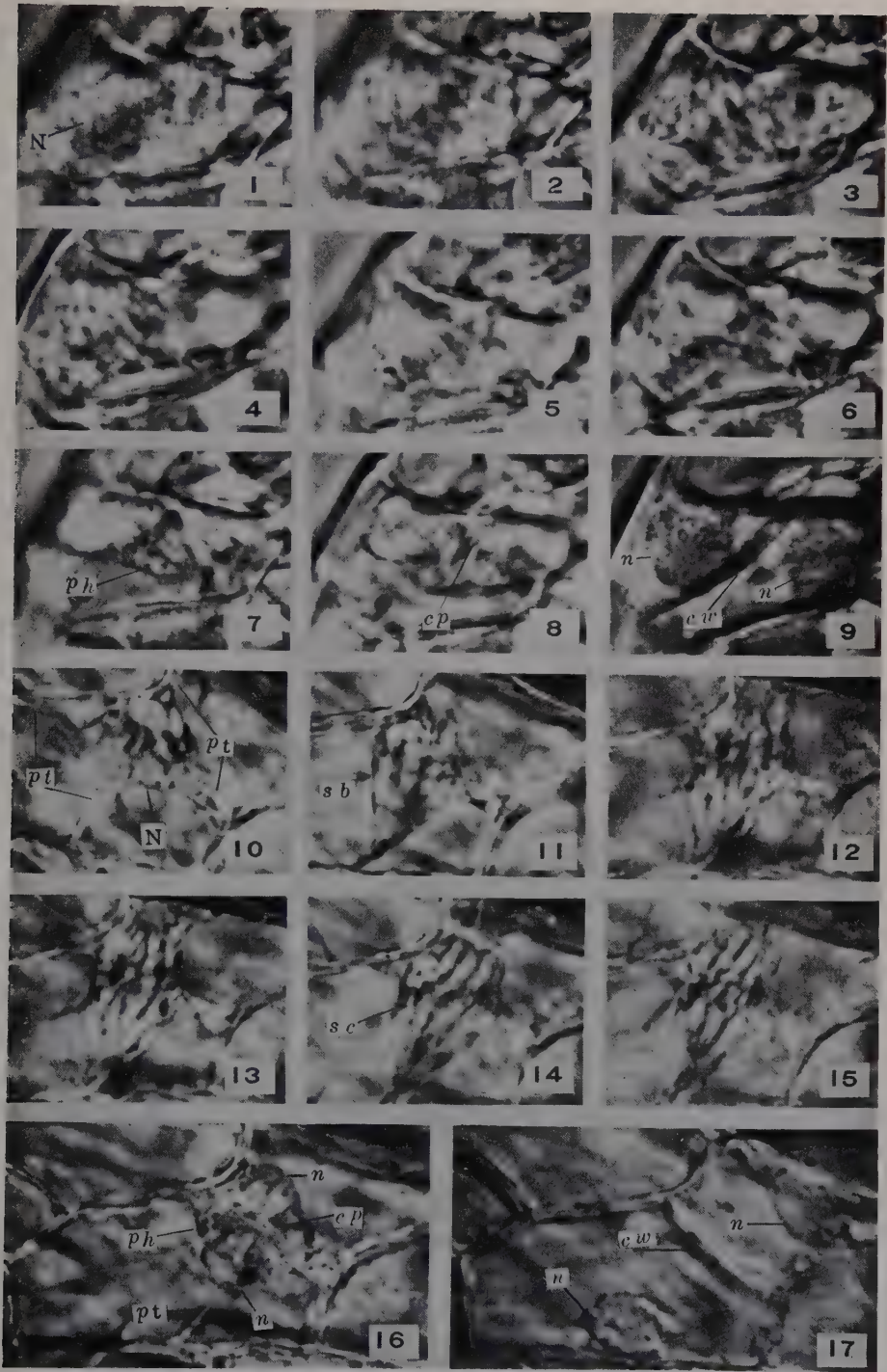
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Investigation on Mitosis *in vivo*

第 1 図 版 説 明

　図 1～9 は *Allium fistulasum* の内花蓋 (*ca.*×1200)，図 10～17 は *Vicia faba* の托葉 (*ca.*×800) の
それぞれ同一細胞における生体核分裂像の連続的変化を示し，原寸のまゝ製版した。

　図の番号の次にあるはすべて撮影時刻を表わす。

図 1～9:　*Allium fistulosum*

1954 年 6 月 10 日 11 時 37 分，8％ 蔗糖を含む 1％ 寒天板処理。15 時，前期のはじめ。

(1)　16:43.　室温 17°C。前期。核内における染色絲の変化。

(2)　17:35.　前期の中頃。染色絲は次第に染色体の形態をとりつゝある。

(3)　19:00.　中期。染色体は中央にならぶ。

(4)　19:12.　後期のはじめ。染色体は次第に極へ移行。

(5)　19:16.　後期。

(6)　19:25.　後期の終り。

(7)　20:25.　終期。隔膜形成体の発達。

(8)　20:50.　終期の終り。母細胞膜の 右側に 細胞板到達，左側はなお発達中。21 時 28 分細胞板は左
　　　　　　へ到達。

(9)　6 月 11 日 10:40.　2 娘核と完成した細胞隔膜。

図 10～17:　*Vicia faba*

1955 年 1 月 31 日 11 時 44 分，9％ 蔗糖を含む 1％ 寒天板処理。15 時 28 分，核の表面にある細胞
質は盛んに活動して数条の細胞質紐が母細胞膜にむかつて放出。

(10)　15:56.　室温 13.5°C。前期。母細胞膜へ到達した細胞質紐発達して太くなる。

(11)　17:10.　前期の終り。細胞質紐によつて核の外形は紡錘体として完成近く，核内では染色体として
　　　　　　の形態的変化がみられる。

(12)　17:36.　中期。染色体は紡錘体中央に位置。

(13)　17:36.　中期の終り。染色体は次第に極へ移行。

(14)　17:43.　後期のはじめ SAt 染色体がみられる。

(15)　17:47.　後期の中頃。

(16)　18:18.　終期の終り。2 娘核の間に発達する細胞板及び 細胞質紐によつて母細胞膜の 右側に発達
　　　　　　する隔膜形成体。

(17)　2 月 1 日 10:22.　2 娘核と完成した隔膜。

N，分裂核。ph，隔膜形成体。 cp，細胞板。 cw，隔膜。pt，細胞質紐。 Sb，紡錘体。 Sc, SAt 染色体。
n，娘核。

ミトリササゲの発芽期における糖

藤 井 良 平*

Ryohei FUJII: Paper-chromatographic Survey of Soluble Sugars
in Germinating Bean Seeds

さきにわれわれ[8] はミトリササゲ幼苗の 各器官が示す種々の 組織成分の含量変化 を報告したが，本報では同じ材料における糖類の質的変化について，ペーパークロマトグラフを用いて追求した結果を報告する。

材 料 及 び 方 法

1952 年秋，本教室の圃場で收穫し約 10 ケ月暗所に貯蔵したミトリササゲ (*Vigna sesquipedalis*) 種子を材料として用いた。予め 30℃ の水に 5 時間浸漬した種子を 30℃ の暗所で砂耕し，播種後 5 日目までの幼苗を実験に供した。

幼苗を子葉，幼葉，胚軸，エピコチール，及び幼根の 4 器官に分離し，それぞれの適当量に海砂及び 80% 酒精を加えて磨砕したものを冷蔵庫内で一晩放置した。遠心分離上澄を 40℃，減圧下でシラツプ状にまで濃縮し，その中に存在する糖をピリジン（時により水）に転溶させ，その適当量を一次元ペーパークロマトグラフに使用した。

試みた展開剤は 1) 20% 含水フエノール，2) *n*-ブタノール-ピクリン酸-水 (80 ml.: 2 gm.: 20 ml.), 3) tert-ブタノール-ピクリン酸-水 (80 ml.: 2 gm.: 20 ml.) 及び 4) *n*-ブタノール-醋酸-水 (4:1:2) [12,13] の 4 種類である。使用紙：東洋濾紙 No. 50; 温度：室温；展開時間：約 20 時間；上昇法。

試みた発色剤は 1) レゾルシン-塩酸[2], 2) ベンチジン-三クロル醋酸[1], 3) アニリン-フタール酸[11] 及び 4) アムモニア性硝酸銀溶液である。

実 験 結 果

第 1，第 2 及び第 3 図は水に 5 時間浸漬した

* 名古屋大学理学部生物学教室

種子，ならびに播種後 2 日目及び 5 日目の幼苗について得られた遊離糖のペーパークロマトグラムを示す。発芽期を通じていずれの器官にも共通して検出されるサツカロースのあらわすスポツトの面積が，大体一定になるよう試料の使用量を加減し，どの糖についてもスポツトの面積を比較することにより，サツカロースに対する相対濃度の高低を推測することが出来るようにしてある。

含水フエノール，レゾルシン-塩酸 の 組合せで得られたペーパークロマトグラム（第 1 図）で認められる 4 点のうち，Rf 値 0.39 をしめる小豆色のスポツトは Rf 値，又は対照クロマトグラムとの比較からサツカロース又はグルコースと同定されるが，後者は レゾルシン 塩酸液によつて呈色反応を示さないことが知られている故，一応サツカロースと推定される。Rf 値 0.51 の同じく小豆色に鋭敏に呈色するものは同様な方法でフラクトースと同定出来る。フラクトースのスポツトの上縁に接する部域と，Rf 値 0.82 の部域に，試みられた発色剤の うち レゾルシン-塩酸液によつてのみ呈色（青色）する 2 つの スポツトを認めた（以下，前者を X, 後者を Y 物質と略記する）。

フエノールで展開したものをベンチジン-三クロル醋酸液で発色せしめると（第 2 図），グルコースは特長的な暗褐色を呈し，フラクトースとサツカロースとは黄褐色を呈する。このさい，Rf 値 0.39 の暗褐色スポツトはレゾルシン-塩酸液の場合に比較してより大きな面積を占める。この事実からすると該域はサツカロース以外にグルコースをも含むと考えるべきであろう。

展開剤として *n*-ブタノール-ピクリン酸-水を，発色剤にベンチジン-三クロル 醋酸を使用した場合（第 3 図），第 2 図に示す実験によつて不可能であつたサツカロースとグルコースとの分離は行
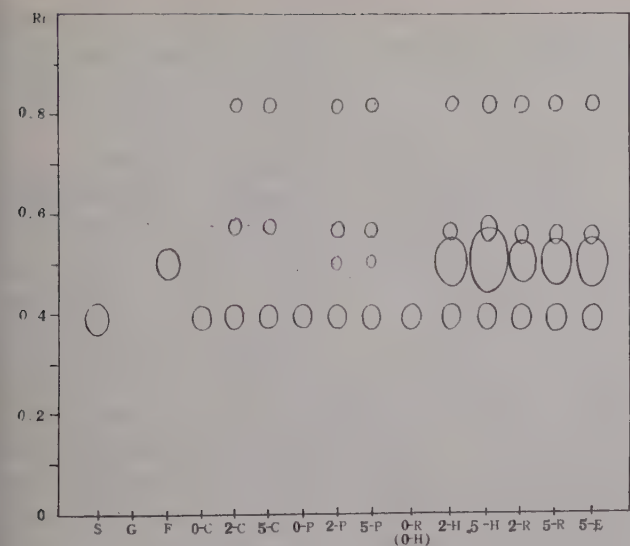
Fig. 1. Paper-chromatogram of alcohol extracts of seed embryo tissues of *Vigna sesquipedalis* germinated in the dark. Developer: 80% phenol solution. Developing conditions: 20 hrs., room temperature. Spraying reagent: resorcinol-HCl. S, G, and F; and C, P, E, H, and R denote authentic sucrose, glucose, and fructose; and cotyledon, plumule, epicotyl, hypocotyl and radicle, respectively: figures attached to the letters signify the age of germs from which the tissues were excised; e. g. O-C and 2-P mean the cotyledons from O-day-old and the plumules from 2-day-old germs, respectively.
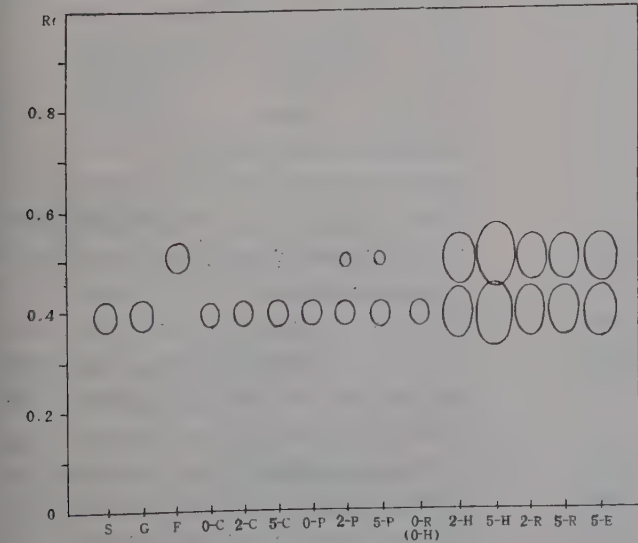
Fig. 2. Paper-chromatogram of alcohol extracts of seed embryo tissues of *Vigna sesquipedalis* germinated in the dark. Developer: 80% phenol solution. Developing conditions: 20 hrs., room temperature. Spraying reagent: benzidine-trichloracetic acid. See Fig. 1 for symbols used.

われるが，X と Y 物質は確認出来なかつた。

　なお，図示せる例以外 の 展開剤，発色剤を用いた場合にも，かくべつ異質の結果は得られなかつたから記載を省略する。

　さて，これらの結果から次の事実が判明する。発芽期のミトリササゲで認められる遊離糖ないし糖様物質はサツカロース，グルコース，フラクトース，X 及び Y 物質である。しかも子葉では浸漬直後（0 日目）より播種後5 日目に到る全期間，また幼葉を含む軸性組織では 0 日目において，いずれもフラクトースの存在が認められない。X と Y 物質は 0 日目ではまだいずれの器官にも検出されず，発芽が進むとともにすべての器官に出現する。幼葉以外の軸性器官では，発芽の進むに従つてグルコース及びフラクトースの顕著な生成がみられるが，これに反し幼葉では調査全期間を通じてフラクトースの生成はあまり著しくない。

　X と Y 物質について次の如く今少し詳しく検討したが，結局同定には到らなかつた。

　播種後2日目の子葉を材料として，方法の項で述べた原抽出液（最後に水に転溶させたもの）を多量に作り，これをフエノールを用いて濾紙上に帯状に展開させた。次いで濾紙の左右両辺を切取つて レゾルシン−塩酸液にて青色に発色する部域 を 定める。残りの濾紙から該当部域を切取り，メタノールで抽出し，減圧濃縮した後水を加え，これに溶解する物質について検討した。

　フエノールで展開したペーパークロマトグラム上で，X と Y 物質の示す Rf 値はそれぞれ 0.50 と 0.82 であつて，ペントースである可能性が考えられる故，キシロース，アラ
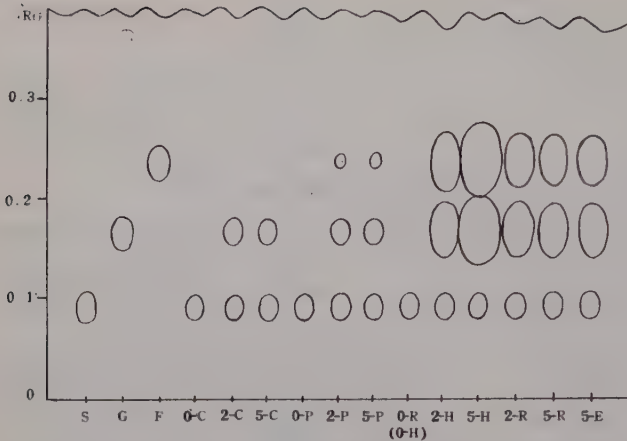
Fig. 3. Paper-chromatogram of alcohol extracts of seed embryo tissues of *Vigna sesquipedalis* germinated in the dark. Developer: *n*-butanol-picric acid-water (80 ml.: 2 gm.: 20 ml.). Developing conditions: 20 hrs., room temperature. Spraying reagent: benzidine-trichloracetic acid. See Fig. 1 for symbols used.

ビノース，及びリボースと共に展開してみた。第 4 図より明らかな如くいずれも Rf 値をことにする。又レゾルシン-塩酸液による呈色状況もことなり，X と Y 物質はいずれも青色であるのに対し，キシロースは灰青色，アラビノースとリボースはウグイス色に発色する。抽出液についてペントース反応たるオルシノール反応を試みたが，結果は負であつた。なお，同じ抽出液についてジフェニールアミン反応を試みたが，結果はやはり負であつたから，問題の物質はデソキシペントースでもないと考えられる。

考　　察

ミトリササゲの子葉に貯えられた澱粉は発芽の進行に伴つて漸次減量し，子葉の脱落する6日頃にはほとんどなくなる[3]。澱粉は子葉組織において移動可能な可溶性糖類にまで分解されるであろうが，その場合アミラーゼとフォスフォリラーゼの関与が考えられる。丸尾[7]によればアミラーゼはアズキ種子及びインゲン種子にはなく，ソラマメ種子に僅かに存在するに過ぎないのに対し，フォスフォリラーゼはこれらの材料のいずれにも分布しているという。これらと近縁のミトリササゲの種子でも恐らくアミラーゼは存在せず，澱粉分解は専らフォスフォリラーゼ作用によるものと考えられる。アミラーゼ作用の中間生

成物であるマルトースが本実験において全く検出されなかつたこともこの推測を裏書きするであろう。

発芽期の子葉が多量のサッカロースを含有している事実は，澱粉分解産物たる六炭糖が更にサッカロースに変化させられることを教える。このサッカロース生成は発芽期の分解的性格の強い子葉組織におけるおそらく例外的な合成反応として極めて注目すべきものと考えられる[8]。この観点に立つてわれわれは子葉のサッカロース合成を解析し，すでに 2，3 の知見を得ている[3, 10]。

高等植物における糖の移動形は常サッカロースであるとされて通いるが[4, 6, 14]，子葉におけるサッカロース合成の意味もまたこの線に沿つて解されるであろう。

子葉内でフラクトースが全く認められない事実はインヴェルターゼの不在，或は優先的なフラクトースの利用を予測せしめる。サッカロースの加水分解産物のうち，安定なグルコピラノースに先んじて，不安定なフラクトフラノースが消費されるならば[5]，インヴェルターゼが存在するとしてもフラクトースが蓄積しないことはあり得よう。

幼葉を除いた軸性器官ではサッカロースに比し多量のグルコースとフラクトースが存在しているが，それらは子葉から運ばれて来たサッカロースが，恐らく組織の含有するインヴェルターゼ作用をうけた結果とみられる。

これに対して幼葉では，0 日目にはみられなかつたフラクトースが発芽の進行と共に漸次出現するが，その量はサッカロースに比して少い。この点で同じく合成的組織の名で一括されるとは云え，幼葉が他の軸性器官と著しい対照をなし，むしろ子葉的な代謝パタンを示すことに注意すべきであろう。元来幼葉と子葉は相同の器官であるから，両者が共通の代謝系を有する可能性は大きいにちがいない[9]。

Wanner[5]もエンドウ幼苗で著者とほとんど同様な事実を得ている。
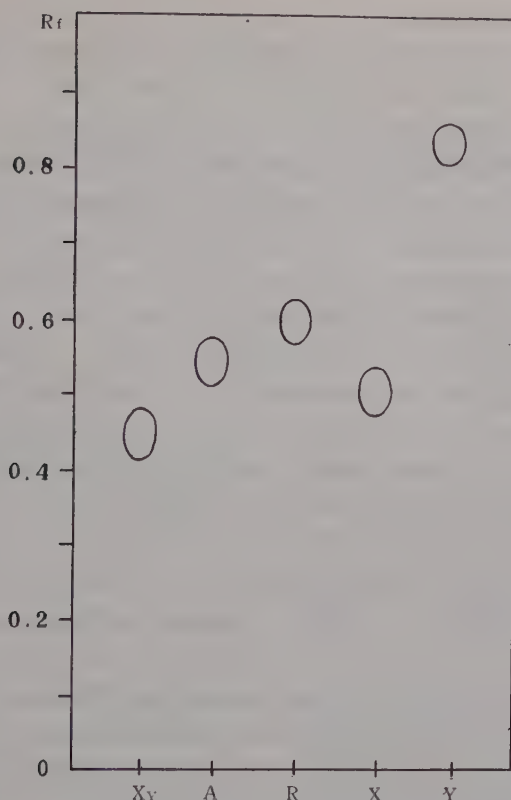
Fig. 4. Comparison between paper-chromatograms of several authentic pentoses and of 'X' and 'Y' substances obtained from the cotyledons of 2-day-old germs. Developer: those 80% phenol. Developing conditions: 20 hrs., room temperature. Spraying reagent: resorcinol-HCl. Xy: xylose, A: arabinose, R: ribose, X: 'X' substance, and Y: 'Y' substance.

要　　約

　暗所で発芽させたミトリササゲ種子における可溶性遊離糖の分布について行つたペーパークロマトグラフ分析から次の様な事実を知つた。

　1.　発芽前の種子で一般的に認められる糖はサツカロースであり，グルコースとフラクトースはどの組織にも全く検出されない。

　2.　幼葉，幼茎（胚軸及びエピコチール），及び幼根の各器官では発芽と共にサツカロース，グルコース及びフラクトースが認められる；なおレゾルシン-塩酸液に対し青色に発色する未同定の糖

様物質種 2 が認められる。

　3.　幼茎と幼根ではサツカロースのほか，多量のグルコースとフラクトースとが存在するが，幼葉のフラクトースの含量は例外的に少ない。

　4.　子葉では全発芽期を通じてサツカロースが多量に存在し，少量のグルコースがこれに伴う。しかしフラクトースは全く認め得ない。

　指導して頂いた太田助教授，試薬リボースを分与された山田教授及びオルシノール反応，ジフェニールアミン反応の試験を願つた大沢君に厚く御礼申し上げる。

Summary

Sugars contained in the alcohol extracts of seed embryos of *Vigna sesquipedalis* germinated in the dark were studied semi-quantitatively on onedimentional

ascending paper-chromatograms.

No other sugar than sucrose, glucose and fructose was detectable, excepting that two unidentified blue-colored spots of relatively high Rf values were made appeared when resorcinol-HCl was sprayed. These sugar-like substances behaved different on paper-chromatogram from galactose, mannose, rhamnose, xylose, arabinose and ribose; they also did not react with the diphenylamine as well as the orcinol reagents.

The seeds before germination contained only sucrose, no hexose being detected. As germination proceeded, glucose and fructose were formed rapidly to predominate soon over sucrose in the epicotyl, hypocotyl and radicle. In plumules, on the contrary, the amount of glucose and fructose did not exceed that of sucrose; the production of fructose in particular was very much small. The cotyledon, as a homologous organ to the plumule, showed a similar sugar pattern to that of plumules, i. e. a bulk of sucrose accompanied by only a scanty amount of glucose and no fructose.

In the cotyledon tissues maltose was absent, and, therefore, the starch degradation might not be performed by the action of amylase but likely by the phosphorylase action instead.

These results would suggest, in accordance with Wanner (1952), that in the cotyledon sucrose may be synthesized from the products of starch degradation, hexoses, and transported as such into the growing parts of the germs to be split again to free hexoses probably by the action of invertase.

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ミ ル の 生 殖 と 体 成 形

新崎盛敏*・德田　広*・藤山和惠**

Seibin ARASAKI, Hiroshi TOKUDA & Kazue FUJIYAMA:
The Reproduction and Morphogeny in *Codium fragile*

緒　言

　ミル属 *Codium* は非細胞構造 coenocytic form
をとるミル目 Siphonales の代表者で横壁のない
糸枝がからみあう特異な体構造は古くから研究者
が注目した。体内部の枝は細く，分岐しげく，無色
で，不規則にからみあつて髄部をつくるが，表層
部では糸端が膨大し，葉緑粒を貯えて胞囊 utricle
になり柵状に整列する (fig. 1)。体の外形，糸枝
や胞囊の形態等は種別の特徴になるので詳しく調
べられており，近時は従来の分類に再検討を要す
るとて益々微細な点まで調べられる傾向にある
が，然し未だどんな過程をへてミル体が造られる
かについては詳細な報文がない。その繁殖法につ
いては，無性胞子がなくて有性の配偶子のみがあ
り，しかも配偶子には雌雄でいちぢるしい大小の
差があつてそれぞれ別の子囊内に造られる事は
Thuret (1850) をはじめ多くの研究者が確認して
きた。細胞学的研究は Williams ('25)，Schussnig
('29, '50) のがあり，本体が 2*n* (n＝10 または
20) で子囊内で配偶子形成時に減数分裂があると
の一致した見解を報ずる。然し雌雄配偶子囊の現
われ方については研究者によつて異なる観察が報
ぜられ，雌雄同株とか異株とか云われ実験種を異
にする点もあろうが一致した見解が得られていな
い。特に近時 ('50) Schussnig と Hartmann &
Haemmerling とが夫々別々の観点，方法で進め
ていた 20 余年の長期にわたる研究結果を報ずる
が，両者の見解も異なる。核学的研究を詳細に行
つた Schussnig は性分化の本質にまで及ぶ考察を

した後，配偶子形成時に内因的に性決定があると
するのに対し，Hartmann & Haemmerling は多
数の植物体を長年調査して表現的性決定 pheno-
typic sex determination があるものとする。
　邦産ミル属についての分類学的研究は之までに
も多いが，生殖や体形成過程等については報文が
殆んどない。新崎は 1940 年以来邦産のミル属特
にミル *C. fragile* Hariot を主としヒラミル *C.
latum* Sur.，ハイミル *C. adhaerens* Ag.，イモセ
ミル *C. tomentosum* Stackh. 等の生殖と体形成
を追究中である。未だ不明な点が多いが，ミルに
ついては従来未知の新知見も得られたから，玆に
伊勢三河産で 1949 年までに得られた結果と，藤
山・德田両君と共に東京湾内や神奈川県三崎付近
産で 1951～53 年間に得られた結果とをまとめて
報ずる。
　観察と実験　ミルは太平洋沿岸に広く分布し，
日本国内でも殆んど全沿岸に産する。特に波静か
な内湾，中でも外洋水の影響を強くうける区域に
多産する。本州の関東東海地方では干潮線辺から
15 m 位の深処までにあり，岩礁，小石，杭上または
他海藻上等に着生する。幼体は初冬頃から現われ
始め晩春から初秋にかけて生育旺盛で，晩秋頃か
ら体勢次第に衰え折からの荒天風浪のため汀に打
あげられて死ぬのが多くなり，真冬には最衰期に
はいるが，生き長らえて越年するのもあり，殊に
深処にある物では越年者が多い。なお初夏頃から
初冬頃まで，場所によつては真冬の頃までも配偶
子囊が見られ，特に秋以後は殆んどすべての成体
が子囊を持つている（第 1 表）。
　配偶子囊は胞囊上に特別な分枝として 1～3 ケ
生じ，德利形または卵形で，0.2～0.3 mm の長径
を有して肉眼でも認められ得る程である。幼期の

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　* 東大農学部水産植物学教室
　** 広島曉の星女子学院

Table 1. Sex differentiation in *Codium fragile*

| | Ise Mikawa Bay ('40~'43) | | | | Tokyo Bay ('51~'53) | | | |
	sterile	⚥	♀	♂	sterile	⚥	♀	♂
Jan.	25	0	0	0	5	0	2	0
Febr.	32	0	0	0	5	5	2	5
Mar.					14	2	1	
Apr.								
May	51	3	63	92				
June	50	2	3	9	16	0	8	0
July	21	0	9	12	79	10	107	4
Aug.	33	1	27	21	15	3	71	4
Sept.	8	15	192	170	7	0	108	40
Oct.	0	0	4	2	3	4	7	1
Nov.	0	2	13	14	4	16	16	5
Dec.	0	0	2	2	6	22	26	3
total	220	23	313	322	154	62	348	62

子嚢では雌雄の区別が至難であるが熟するにつれて相違が次第に明かになり容易に識別できる様になる。雌性嚢は上半部がやや広く，大粒の緑色粒（雌配偶子）に充たされるが，雄性嚢は上半部やや細く，微小な黄緑色粒（雄配偶子）に充たされる（fig. 2, 3）。

　子嚢はミル体のどこと限らず任意の体部に造られ，しかも熟度の違う種々の形態の子嚢が同時に多数見られるが，出現初期頃は体下半部特に分岐部付近から多く造られる傾向がある。雌雄異株体も同株体もあつて，それ等の出現状態は時期，採集地等で多少違うようだが，全体的にみると異株体が多く，かつ雌株と雄株とは大体同数位であると云つてよかろう（Tab. 1）。なお欧米の諸研究者は雌先熟と云うが邦産のミルでは必ずしもそうとも云えぬ様であり，また実験的に未熟体片や雌雄の子嚢をすでに持つ体片を籠に入れ海中に吊して1～2ケ月の後における変化状態を調べたが（実験中颱風等のために死者，流失者があつて不備な処があり再検討を要する点はあるが）性分化過程，性転換等に関して規則的な現象はないらしい事がうかがわれた（Tab. 2）。また太西洋，地中海産

の *C. decorticatum* で Schmidt ('28) は同一胞嚢上に雌雄の子嚢が同時に作られる例を報じたが，筆者等の観察ではそんな場合はみられなかつた。

　完熟期になると両性の子嚢ともに頂端が少しく突出し，頂点から嚢内に通ずる溝道が通じて無色の粘質物に充たされる。嚢内の配偶子は溝道を1ケづつあがつてきて，内圧で出来る頂点の開口から，噴出する粘質物と共に嚢外に放出される（fig. 4）。両配偶子とも頂生する2本の鞭毛を有し游泳力があるが，形態的には雌雄で相違が大きい。雌配偶子は丸味ある紡錘形で 19.0～30.0μ×12.5～21.0μ（休止球の直径，平均 14.5μ）の大きさを有し，体内葉緑粒に富むが眼点を欠き，游泳力は活溌でない（fig. 5）。雄配偶子は長めの紡錘形で 5.0～8.8μ×2.4～4.0μ（休止球の直径，平均 4.4μ）の大きさしかなく，微小の緑粒を2～3ケ持つだけで黄白色を呈し，眼点もないが活溌に泳ぎ廻る（fig. 6）。両者とも殆んど光に対する走性を示さずに游泳し，接合能力は運動力のあるものにだけあるらしく，静止して球形になつた雌配偶子はすでに接合力を失つて了つていた。1

Table 2.　Diagram showing the flexible properties of sexuality of *C. fragile*

condition in start (1952, July 11)	plants in start	4 weeks after (Aug. 9)				8 weeks after, (Sept. 9)			
		ster.	⚥	♀	♂	ster.	⚥	♀	♂
sterile.	17	8	0	6	3	11	2	1	1
♀	10	11	0	0	0	9	0	0	0
♂	3	2	0	1	0	1	0	2	0

(1952, Sept. 22)	plants	* 3 weeks after, (Oct. 11)			
		ster.	⚥	♀	♂
sterile.	7	5	0	1	1
♀	59	8	2	25	1
♂	40	3	3	0	18

* Owing to a heavy storm on Oct. 6, most culture plants were drifted away.

ケの雌配偶子の周囲に数ケの雄配偶子がとりつく事もあつたが接合は1対1で行われ，接合点は雌配偶子の体位上定まつた処はない様である (fig. 7)。

接合子は間もなく鞭毛を失い，静止して丸まり，周囲に膜を生じて発芽し始める (fig. 8, 9, 10)。接合し得なかつた雌雄配偶子は放出後 2〜3 時間内に静止して丸まるが外膜を生ぜず，1〜3 日で腐死する場合が殆んどで単為発芽する物はみられなかつた（雌では極僅少例で単為発芽らしいのがみられることもあつた）。

発芽し始めた接合子は次第に容積をまし，やがて細長くなる物，縁辺に虚足状の突出瘤を出す物等形態に変化多いものになる。然しずつと分裂は起らず体内には隔壁が出来ない。やや成長すると発芽体は単管状に伸びてゆくが，伸長部は少し細くなり色素粒少ないのに対し，根部では膨大したり不規則に瘤状突起を生じ，また内部は色素粒に富むようになる等上下の分化がみられる。成長しても根部では膨大し突起瘤を増すだけで余り変化がないけれども，伸長部では伸長してゆくと共に次々と側出する分枝を沢山出して (fig. 11, 12)，連基的分岐 sympodial branching をするフサフサした叢生体になるが，体糸中どこにも横壁が造られない (fig. 12, 13)。体糸中には葉緑粒が充満して鮮緑色を呈し，太さは生育状態等で変化が大きいが大体 30〜50 μ 位で，その外膜は平滑な物が多いが時により細かいくびれが多かつたり，糸端が肥大して根棒状になることもある (fig. 14)。発芽体の生育速度は外囲条件殊に光，温度等に

影響されて不同であるが，大体において接合後1〜2 日で外膜著明になり，一週間後に伸長が始まり，1〜3 カ月後分枝が出始まる。その頃までは成長遅いが後では速くなり，また冬季の低温時はおそいが，春夏の高温時には速い等の傾向がみられた。1942〜46 の 4 年半以上と 1951〜53 の 2 カ年と二度室内静水中で多数の発芽体を長期培養したが，叢生体はフサフサと大きくなるだけで，30cm 以上にもなつたが之等から如何にしてミル体ができ上るかを見ることは出来なかつた。また胞子嚢らしい物を作らす事も出来なかつた。

一方海中での天然におけるミル体出現状況をみるに，12 月頃から 3 月頃まで最干潮線付近に幼体が出てくるが，幼体ではフシナシミドロ *Vaucheria* 状の多分枝の糸状叢生枝がからみあつてマット状になつた緑叢根がさきに発達し，その中から 1 ケまたは数ケの乳頭状体が現われ，直上伸長してミル体になついていく (fig. 15)。なお淡水の影響強く変化はげしい環境と考えられる川口付近等では，こんな緑叢体が 6〜7 月頃まで生残る場合が多い。その中から直立するミル体が出てくるのはみられなかつたが，体糸の状態から上記の緑叢根と同一物とみなされた。更に海中で得たミル体または体枝片を上記の胞子培養と同条件で室内培養すると，体枝がもとの構造のままで成長する事は殆んどなくて任意の枝部特に枝端や切断部から無色または淡緑色の糸状枝が多数新生するだけである。この現象は簡単に起り，すでに Schwartz ('30) が詳しく観察して新生枝の形が外囲条件で
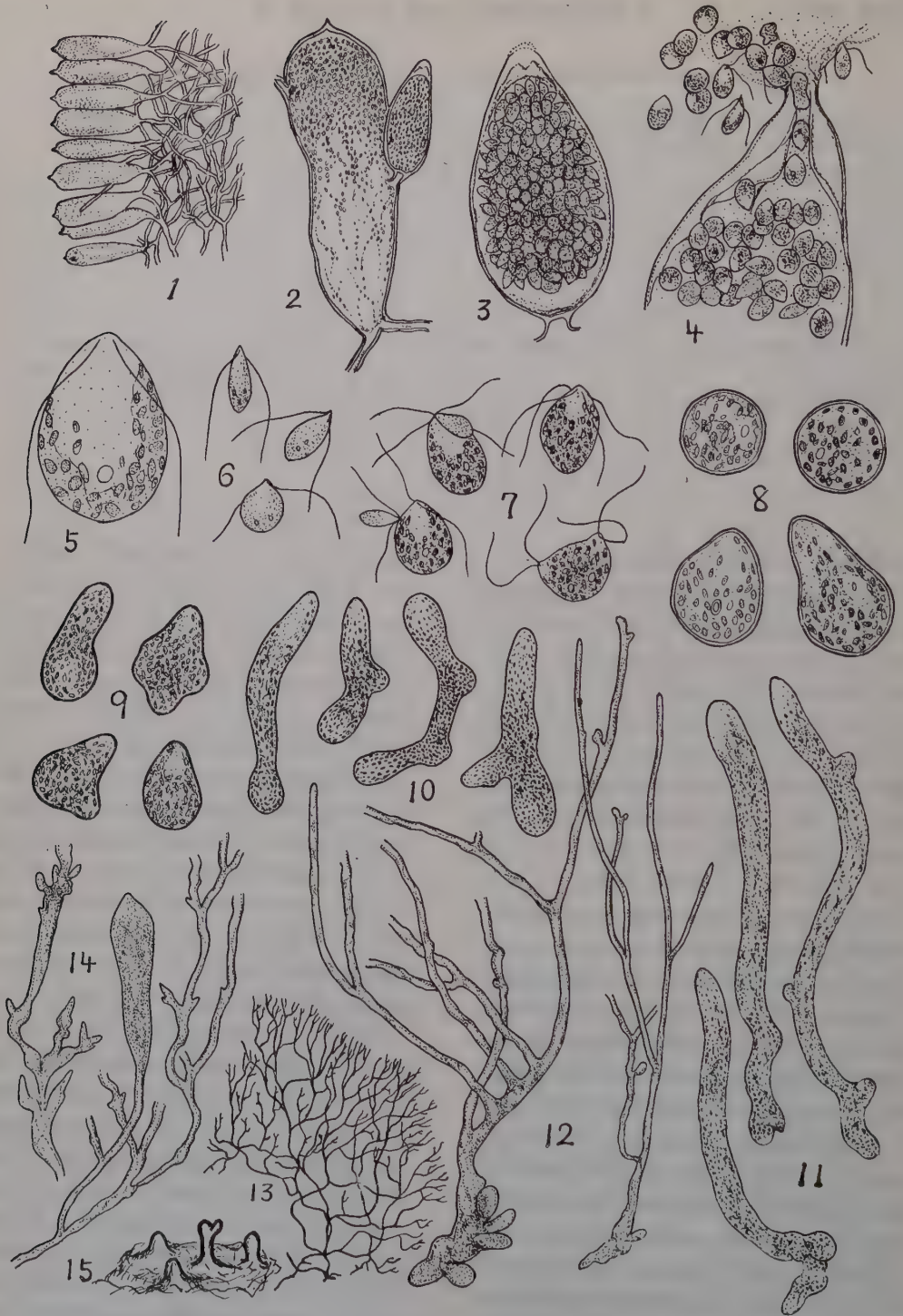
Figure 1.　Reproduction of *Codium fragile*

　　1. part of a longitudinal section (diagrammatic)　2. a single utricule with a gametangium　3. a female gametangium　4. liberation of female gametes　5. a female gamete　6. male gametes　7. conjugation of gametes　8. resting zygotes and their germination　9. germinating zygotes　10, 11, 12. young plants derived from zygotes 13. about 6-months old plant derived from a zygote　14. parts of filament　15. part of basal mat of young *Codium* found in winter in the sea.

変異多い事も指摘している。之等の新生枝は母体
から切離された後も生長らえ，成長を続けて，接
合子から出来た物と区別できぬ叢生体になつた
が，5カ年以上培養し続けても矢張り再びミル体
の構造になることもなく子嚢形成を見ることも出
来なかつた。

　　論議と考察　　海中のミル本体と室内で接合子
から育てた培養体とでは別属，別種の物と思える
程の相違があるが，上述の諸点からみて外囲条件
特に静水と云う異常要因に伴なう悪条件のために
起る変型ではないかと考えられる。それにしても
連基的分岐をくりかえしてフサフサした体形をと
る事はミルの様な構造になるまでの進化過程を考
える際に興味あることと思われる。

ミル体が連基的分岐をする糸状枝のからみあいで
出来ていることには疑いをはさめぬが（fig. 1），
体をつくる際天然では恐らく，初めは水平に拡が
るマット状の盤状根部ができ，やがてその一部に
おいて糸状枝が密にからみあつて直上する本体部
が生ずるのであろう。そして本体では内部にはい
つた枝は無色化し殆んど変形がないのに対し，
表層に出た枝端部は葉緑粒に富む様になりまた膨
大して胞嚢に変形するのではなかろうか。なお胞
嚢の形は種により種々で，単独の物や分岐する物
等あるがその相違は Silva の云う如く体枝のどの
部が変形したかを示す系統的意味を持つものであ
ろう。

　なおマット状の緑叢根や直上する本体を造る際
の構成枝は，同一の接合子からできた叢生体の枝
だけがからみあうのか，または近くで発芽した複
数の接合子に由来したものも一緒にからみあうの
か，上記の実験観察だけでは明らかにすることが
出来ないが，恐らく何れの場合もあり得るのでは
なかろうか。

　若しミル体のでき上る過程が上の様だとすると
ミルの個体には単元的体や複元的体があり，また

複元的体では体部によつて単元的体枝，複元的体
枝の混合状態にも差がある筈である。しかもそれ
等各々の出現様相は不規則で，海水運動による胞
子散布状態また体構成枝の成長状態等にもとづく
複雑な変化多い表現型がみられるであろう。此の
点を考えると Schussnig の云う如く性決定は雌配
偶子形成時に行われて接合子からできる叢生体は
雌雄異株である場合でも，これ等がからみあつて
ミル本体を作る際には複雑な組合せがある筈だか
ら，ミル個体においては性分化の様相が区々で，
雌雄異株体同株体のでかたに Hartmann &
Haemmerling の云う様に表現型と解される現象
が起るのではなかろうか。

　擱筆に当り恩師三宅驥一先生に深謝する。

摘　要

　1. 邦産のミルは初冬から現われ始め春夏に繁
茂し，夏から初冬に雌雄の配偶子嚢を作る。性分
化は雌雄異株体が多く雌雄同数位であり，同株体
も少し混るが，それ等の出現状態は場所，時期に
より相異がある。

　2. 配偶子は雌雄で大小の差があり，接合は運
動中に行われる。接合子は発芽して隔壁のない管
状枝からなるフサフサした叢生体に成る。この叢
生体は海中のミル本体とは全く違つた形態である
が，ミル体の緑叢根の構成枝またはミル体を実験
室内で培養した時に出る新生枝と全く似ている。

　3. ミル体が出来上るのは恐らく上記の様な叢
生体の体枝がからみあつて，初めはマット状の緑
叢根に拡がりやがてその一部で体枝が密にからみ
あい乍ら直上突出して本体を造るものであろう。
その際からみあう体枝が1ケの接合子に由来した
ものだけからなる単元的個体，または複数の接合
子に由来した枝からなる複元的個体もある筈であ
る。ミル体の形成過程がこの様に種々の場合があ
る事が性分化の様相が区々である原因になるので
はなかろうか。

Résumé

1.　The field observations on the vegetation and sexuality of *Codium fragile*
have been made for several years during 1940–1949 and 1951–1953 in the central
region of the Pacific coast of Japan.

　　The young plants begin to appear during early winter and spring, then they
become luxuriant during early summer and late autumn. The reproductive organs
(gametangia) are produced usually from May till December. As for the sexuality,

most plants are dioecious and some are monoecious, while the sexual ratio and the ratio of monoecious to dioecious plants are varied in places or seasons.

2. The gametes are anisomorphic, and the conjugation takes place between a macro (female)- and a micro (male)- gamete. The zygote develops into a tufty plant composed of non-septate, sympodially branched filaments. Though the shape of the free filament is variable, the contents of the filament or chromatophores are identical to those of *C. fragile*. This tufty vaucherioid plant survives over four years becoming much denser, but not into *Codium*-form probably owing to the unfavourable conditions in the culture vessel.

3. From the field and laboratory observations, it is suggested that the frond of *Codium* may be made up by the interweaving of the sympodially branched filaments, producing in the beginning the prostrate basal portion then one or more erect portions. And also it is suggested that these filaments may be originate in a zygote or in a few zygotes: that is to say homogeneous or heterogeneous. If this assumption is acceptable, much variety in sex differentiation in *Codium fragile* seems to be explained by such flexibility in the process of the morphogeny.

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同校職員寮

河原　晨　大阪市立大理工生物・大阪市住吉区
御崎町市営住宅 417 号

日下部有信　京大理植物・京都市北区出雲路神楽
町 1

清水　建美　京大理植・京都市左京区吉田本町 1
田中方

寺田　保　阪大理生物

西村　公臣　奈良女子大理植・京都市伏見区深草
直違橋 6 丁目 280

藤原　健司　神戸大教育学部理科植物学研究室・
神戸市東灘区住吉町赤塚山神戸大住
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中国四国
荒木　精一　山口県光市山口大教育光分校

竹本　博明　広島大理植

谷本土佐雄　高知県中村市県立中村高校・同市中
村町 1303

西尾　隆昌　広島大理植

九 州
小代　基礎　大分県東国東郡国東町県立国東高
校・同町鵜川 1343 の 3 河野方

外 国
姜　悌源　韓国釜山市釜山水産大学増殖学教室

金　仁培　韓国釜山市釜山水産大学増殖学教室

Kondo, K. C.　2457 Liliha St., Honolulu 17,
Hawaii, U. S. A.

住 所 変 更　（昭和30年9月—12月）

北 海 道

小泉　晴一　釧路市宮本町 9 相庭方

吉村　フジ　北大農生物

関 東

下間　実　（近畿より）横浜市南区六ツ川町大
丸 1246 木原生物研究所

鈴木　恕　東京教大理植

野村　克世　世田谷区下馬東京学芸大

北 陸

梅鉢　百子　金沢市横山町 1-41 岡田方

中 部

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東京大学教授　田 宮　　博 監 修
東京大学理学部植物学教室　物質代謝研究会編

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Cytological and Morphological Studies on the Gametophytes of Ferns X

Structures of the Fresh Prothallium and Spermatozoid of Ferns, Revealed by the Polarization Microscope

by Isami IGURA*

伊倉伊三美： 羊歯類の配偶体に関する細胞学的並びに形態学的研究 X
偏光顕微鏡による羊歯類前葉体及び精子の構造

Since the study of Nägeli on the cell membrane, etc. by means of the polarization microscope[4], this method of investigations has been applied to the studies on fine structures in the cells and tissues. In 1931, Hartman[1] used the polarized light in order to detect the presence of cellulose in the gametophytes of *Athyrium filix-foemina* in the course of the microchemical studies. Pfeiffer also by means of the polarization microscope researched on the plant spermatozoids[5] or the mitosis of the cells[6].

Researches made by the polarization microscope are generally used to detect the isotropy or the snisotropy (birefringenece) which is related to cyto- and histo-chemical properties or the micellar structure in animal- or plant-body. In order to make clear the optical anisotropy in the fresh prothallium and spermatozoid-body of some ferns, this polorization-microscopic study was undertaken.

Materials and Methods

The prothallia of following five species were used. Polypodiaceae; *Dryopteris monticola* C. Christensen, *Matteuccia struthiopteris* Todaro, *Rumohra mutica* Ching, and *Thelypteris japonica* Ching: Osmundaceae; *Osmunda japonica* Thunberg. The spermatozoids of *Matteuccia struthiopteris* were also used as materials.

The prothallia cultured in Petri-dishes were dipped in a drop of redistilled water on a slide-glass, then many spermatozoids were extruded out of the ripened antheridia. After that, these prothallia and spermatozoids were observed by means of a polarization microscope (The Japan Optical Co., Ltd. or Reichert). The studies were carried out, in many cases, by the method of orthoscope and the interference colors were researched by the first order red plate (gypsum test-plate). Sometimes the

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* Biological Institute, Faculty of Education, Yamagata University. (Yamagata City). 山形大学教育学部生物学教室. (山形市).

interference figures were observed also by the method of conoscope. The anisotropic properties were confirmed by the observations at the extinction position and the diagonal one.

Results

1. Polarization effects by the crossed Nicol's prisms

Some structural elements of the prothallium were distinctly bright and some were more or less bright and others were dark in the dark field of the crossd Nicol's prisms.

Anisotropic elements are dark when their two axes (directions) of optical transmission agree with those of polarizer and analyzer, namely are dark at the diagonal position. On the other hand, the isotropic elements were always dark

Table I.　The optical properties of structural elements of the
fern-prothallium and spermatozoid

Prothallium	Polarization effects	Optical properties
Prothallial cell		
membrane	＋＋＋or＋	AN
cytoplasm	—	IS
nucleus	—	IS
plastid	±±	very weak AN
Protonema cell	＋＋＋or＋	AN
Antheridium		
outer wall cell membrane	＋＋	AN
inner wall cell membrane	±	weak AN
Spermatogenous mass	—	IS
Spermatid	±±±	very weak AN
Spermatozoid		
border-brim		
nucleus	—	IS
cilium		
cilia-bearing band	±±±	very weak AN
Archegonium (young)		
outer membrane of the neck cell	＋＋	AN
egg	—	IS
Rhizoidal cell		
membrane	±	weak AN
cytoplasm	—	IS
Glandular hair (cap)	＋＋＋	AN
Remarks:		
Sporophyte (leaf)		
cell membrane	＋	AN
cell membrane of the guard cell of stoma	＋	AN
cell wall of vascular bundle	＋	AN

Foot-note: ＋, bright; ＋＋, ＋＋＋, strong bright; —, dark; ±, weak bright; ±±, ±±±, very weak bright.　AN, anisotropic; IS, isotropic.

either at the extinction position or at the diagonal one. The observed results of the optical properties on the structural elements of the prothallium and spermatozoid are as shown in Table I.

a) **Prothallium.** The structural elements which are strongly anisotropic are the membranes of prothallial cells (figs. 14, 15), protonema cells (fig. 16), outer cell walls of the antheridium (figs. 14, 15), and cap of the glandular hair. Although the cytoplasm or the nuclei of prothallial cells have isotropic properties, the plastids have somewhat anisotropic ones. The outer membrane of the neck cell of archegonium is also anisotropic, and the membane of rhizoidal cell is weakly anisotropic.
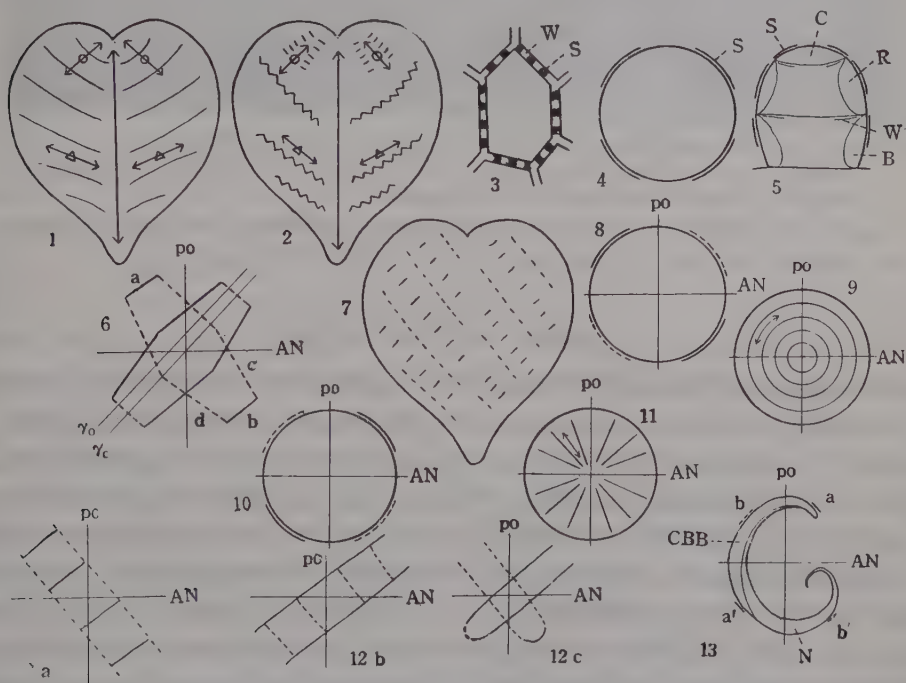
Figs. 1–13. The schematic figures of the prothallium and spermatozoid of fern, showing the effects by means of polarization microscope. PO, Polarizer axis; AN, Analyzer axis; ——— blue, ------ yellew, (Interference color).
1. Three axes of the prothallium; ←→Longitudinal, ←A→Tangential, ←⊖→Radial. 2. The directions of the strong anisotropy in the whole area of prothallium. 3. The segmental anisotropy of a single prothallial cell. S, Strong anisotropic protion; W, Weak anisotropic portion. 4. The upper view of the antheridium which shows the anisotropy. s Strong anisotropic portion. 5. The lateral view of the antheridium which shows the anisotropy. S, Anisotropic; W, Weak anisotropic; C, Cap cell; R, Ring cell; B, Basal cell. 6. Single prothallial cell shown the interference colors. 7. The directions of the interference colors shown, in the whole area of prothallium. 8. The upper view of the antheridium which snows the interference colors. 9. The upper view of the circumferential optical axes in the antheridium. 10. The cap of glandular hair which shows the interference colors. 11. The radial optical axes in the cap of glandular hair. 12. The interference colors of the protonema cell and rhizoidal cell. a, b, Protonema ceil; c, Rhizoidal cell. 13. The interference colors of the spermatozoid. Cilia are not shown. CBB, Cilia-bearing band; N, Nucleus; a, a', Blue; b, b', Yellow (b' is doubtful).

b) **Spermatid and spermatozoid.** The spermatogenous mass contained in the antheridium is isotropic but the spermatids are very weakly anisotropic. While border-brim, nucleus, and cilium of the spermatozoid are isotropic, the portion of the cilia-bearing band is very weakly anisotropic.
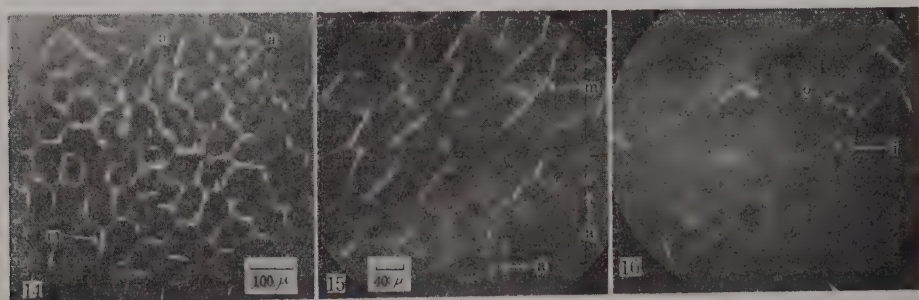
Figs. 14, 15. The polarization effects of the prothallial cell membranes (m), and the antheridia (a) in which the spermatids are contained. 14. *Matteuccia struthiopteris* Todaro; 15, *Dryopteris monticola* C. Christensen.

Fig. 16. The polarization effects of the filamentous protonema cells in *Matteuccia struthiopteris* Todaro. i, Inner membrane; 0, Outer membrane.

2. Intensity of the anisotropy

a) **Whole area of the prothallium.** The membrane of prothallial cell, generally, shows strongly the anisotropic property in every portion of the prothallium. At the central meristematic portion, however, shows the whak anisotropic property. Polarity or gradient of the anisotropy seems not to be shown. b) **Prothallial cell.** The membrane is distinctly anisotropic. The author can presume three axes according to the orientation of development and growth of the prothallial cell (Fig. 1), because he has already considered three polarities in the whole portions of the prothallium in the preceding report[2]. In general, the brightness, that is, the anisotropy is strong in the membrane of the prothallial cell which is nearly parallel to the tangential axis (Fig. 2, 14, 15) and is perpendicular to the radial axis (Fig. 2). In the longitudinal axis, the degree of brightness is not clear. In some cases the brightness was not continuous but segmentary (Fig. 3, 14). c) **Antheridium.** The outer membrane of the peripheral wall (cap cell, ring cell, and basal cell) is bright and shows the anisotropic property. As is shown in Fig. 4, 14 and 15 the anisotropic properties were observed at four portions. The lateral view is shown in Fig. 5. In this case the brightness is more or less weaker than the case of the upper view. d) **Spermatid and spermatozoid.** The results were stated above. e) **Filamentous protonema cell.** The membrane only is anisotropic and the anisotropy of the outer membrane is weak and that of the inner one is strong (Fig. 16). f) **Archegonium.** The outer membrane of the neck cell is anisotropic, whereas the egg is isotropic. g) **Cap of the glandular hair.** This membrane is somewhat strongly anisotropic. h) **Rhizoidal cell.** The anisotropy of the membrane is fairly

weak and that of the young membrane seems to be stronger than that of the old.

3.   Interference color

Fresh prothallia or spermatozoids were brought to focus between the crossed analyzer and polarizer and the position was decided at which the maximum brightness of them were observed.   Then the first order red plate was inserted in the slot, which was put at the diagonal position.   After these treatments the interference colors were observed.

a) **Prothallial cell.**   As shown in Fig. 6, the a- or b-membrane (the membrane which is parallel to the minor diameter of the cell) shows blue color and c- or d-membrane yellow (positive anisotropy).   When the stage of the microscope is rotated about 90°, the colorations of membranes are changed, that is, blue is changed into yellow and yellow into blue (Fig. 6).   In the whole portion of the prothallium, generally, the membrane shows blue color in the perpendicular direction to the tangential axis of the prothallium and appears yellow in the parallel direction to it (Fig. 7).   b) **Antheridium.**   At the position which is angled 45° to the polarizer axis the membrane reveals blue color, and at the position which angles –45° it shows yellow (Fig. 8).   It may be deduced that the direction of the optical axes (micellar arrangements) are circumferential (Fig. 9).   By the 45° rotation of the stage, the blue and yellow colors are changed into red and moreover when the stage is rotated 45°, the portion which was formerly blue shows yellow color and *vice versa*.   c) **Cap of the glandular hair.**   In this case, the relation of blue and yellow is reverse to that in the case of the antheridium (negative anisotropy) (Fig. 10).   This phenomenon may support the conclusion that the optical axis is radial (Fig. 11). d) **Protonema cell and rhizoidal cell.**   As the prothallial cell, the outer membrane of protonema cell and rhizoidal cell show blue color and the inner one of protonema cell yellow in the direction of slow axis of the first order red plate.   In the direction of fast axis of the plate, the outer membrane of protonema cell and the membrane of rhizoidal cell are yellow and the inner one of protonema cell is blue (Fig. 12).   e) **Spermatozoid.**   The spermatozoid whose spiral was destroyed was observed.   The ciliabearing band (cytoplasmic portion) seems to show the interference color as drawn in Fig. 13 (but b′ is doubtful).   As in the case of the cap of glandular hair, the portion a and a′ are blue, and b and b′ are yellow (negative anisotropy), but b′ is doubtful.

4.   Interference figure

The interference figures of the anisotropic elements of the prothallium by means of the conoscope have not been demonstrated so clearly that the result concerning this phenomenon will not be described here and the further studies on them must be left in future.

Discussion and Conclusion

Of the structural elements of fresh prothallium, the membrane of prothallial cell shows the strong anisotropy and outer membrane of antheridium, archegonium, and cap of the glandular hair are also anisotropic. The plastids in the prothallial cell and the membrane of rhizoidal cell show weak anisotropic phenomena and the other cellular elements are isotropic.

On the whole, the principal component of the cell membrane of Pteridophyta is cellulose[7]. Therefore, the degree of the anisotropy may depend upon the micellar arrangement of cellulose or, as Ohara[4] stated, upon the other components such as pectin or lignin which disturbs the anisotropy, in the fern-prothallium too. Accordingly, the distribution of anisotropy in the prothallium is able to be presumed.

When the first order red plate and the crossed Nicol's prisms are used, the membrane of prothallial cell and so on show blue or yellow color. It is considered that in the portion of the membrane which show blue color the slow or γ axis of optical transmission of the membrane is parallel to that of the first order red plate, and the anisotropic structure is thought to be positive in relation to the direction of minor diameter of the prothallial cell. On the other hand, the portion of yellow color possesses the slow axis of optical transmission which are perpendicular to that of the plate and the anisotropic property is negative. As mentioned previously, it may be deduced that the direction of optical axis (micellar arrangement) is circumferential in the cell wall of antheridium and it is radial in the cap of glandular hair.

The spermatids are thought to be very weakly anisotropic, and the spermatozoids are almost isotropic, but the cilia-bearing band of spermatozoid shows the very weak anisotropy and the result of Pfeiffer[5] gave one basis to this result.

Summary

The observations which were made on the fresh prothallium and spermatozoid by means of polarization microscope have made clear the following facts.

1) The membrane of prothallial cell shows the strong positive anisotropic property and the outer cell membrane of antheridium, archegonium, and rhizoidal cell are also positively anisotropic; and the cap of glandular hair is negatively anisotropic. 2) The plastid is very weakly anisotropic but the other structural elements of the prothallium are isotropic. 3) The spermatid and the cilia-bearing band of spermatozoid are very weakly anisotropic. The other structural elements of spermatozoid are isotropic. 4) The polarization effect is thought to depend upon the cellulose or other chemical components and the micellar arrangements of them. 5) Discussions were made also on the interference color.

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Studies on the Development of the Peristome in Musci II.

On the Peristome in *Dicranum japonicum* Mitt

by Shintaro SAITO*

斎藤真太郎：　蘚類の蒴歯発生の研究 II.　シッポゴケの蒴歯について

From the standpoint of the development of peristomes, Cavers[3] divides the Bryales into the following four groups, namely, Tetraphidales, Polytrichales, Buxbaumiales and Eubryales. In the former two groups, the teeth of the peristome are composed of entire cells, while in the latters they are composed of thick-walled cells. And the Eubryales is further devided into the Diplolepideae and Haplolepideae by Philibert[4]. The former with few exceptions has double peristomes, while the latter has single peristome.

In the Diplolepideae the two peristomes are derived from the two concentric layers of cells in the opercular portion of the capsule. The development of the peristome has been described in *Funaria hygrometrica*[5], *Mnium hornum*[6], *Mnium microphyllum*[9] and *Bartramia crispata*[10] among several species of this group. In

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* Biological Institute, Faculty of Literature and Science, Shimane University.
　（島根大学文理学部生物学教室）

these species the outer peristomial layer is composed of thirty-two longitudinal rows of cells and the inner one of sixteen rows, thus, these rows consist of groups, each of which consists of two cells of the outer layer lying opposite one cell of the inner layer in the cross section.

In the Haplolepideae the single peristome is likewise derived from two concentric layers of cells in the opercular portion of the capsule. And as for this group Evans & Hooker[1] published a work on the same subject in *Ceratodon purpureus*, but no further special study on this subject has been followed. In this species the outer peristomial layer is composed of sixteen longitudinal rows of cells and the inner one of twenty-four longitudinal rows of cells, and the cells of the peristomial layers form eight groups, each composed of two rows of cells of the outer layer and three rows of the inner layer. And each of those groups gives rise to two teeth. Consequently, the total number of the teete amounts to sixteen. And in this case each tooth of this group is homologous with the inner peristome of the Diplolepideae.

In the present article, the author deals with the development of the peristome in *Dicranum japonicum*, a member of the Haplolepideae.

Materials and Methods

The materials for the present study were collected by the auther from Sept. 1953 to Oct. 1954, at Matsue, Shimane Prefecture. Bouin's solution was used as the fixing agent. The paraffin sections were cut at 10μ thick and stained with Delafield's haematoxylin.

Development of the peristomial layers

In the present species the opercular segments are divided into quadrants by anticlinal walls. Cross sections through the opercular region of young capsules just below the apical cell show the feature clearly (Fig. 1). This quadrant is the fundamental square and each cell of which is further divided into the outer cells (amphithecium) and the inner cells (endothecium) by the first periclinal walls (Fig. 2). The four cells of ths amphithecium develop into the eight-celled stage by the following anticlinal walls (Fig. 3). And a similar arrangement of the cells can be demonstrated in the early stage of the development of the amphithecial cells divided by periclinal walls, thus giving rise to two amphithecial layers of eight cells each (Fig. 4). In the region of the operculum, successively the stage shown in Fig. 4, the cells of the outer layer are divided by anticlinal walls, thus increase the number of cells to sixteen (Fig. 5). And then the amphithecial layer forms two layers which are made of sixteen cells respectively by the formation of periclinal walls in each cell (Fig. 6) of which the inner layer develops into the outer peristomial layer. The cells of the inner amphithecial layer undergo no further divisions and develop into the peristomial layer. The cells of the outer layer are

further divided by anticlinal walls into thirty-two-celled layer and the endothecium begins to divide (Fig. 7). The cells of the outer amphithecial layer are divided by more anticlinal and periclinal walls. During the increasing of cells in the amphithecial layer, the eight cells of the inner amphithecial layer (Figs. 6, 7), correspon-
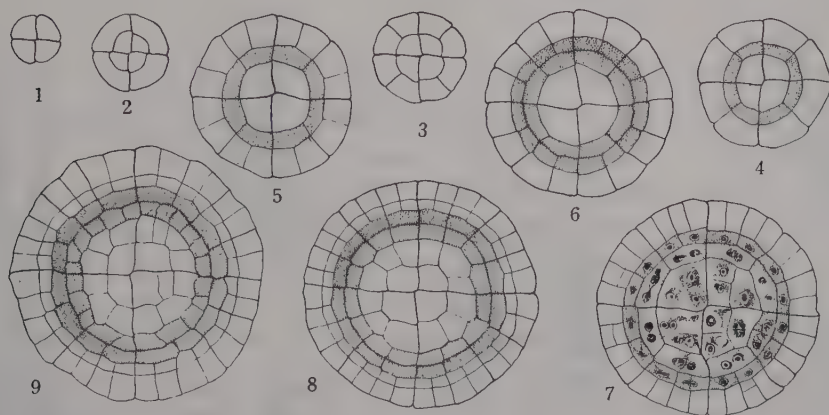
Figs. 1-9. Cross sections through the opercular region of young capsules, showing successive stages of development of peristomial layers stippled × 300: 1. Division of segments to the quadrant or " fundamental square "; 2. Establishment of amphithecium and endothecium; 3. eight-celled stage of an amphithecial layer divided by anticlinal walls; 4. Showing an inner peristomial layer divided by periclinal walls; 5. Division of cells in outer amphithecial layer by anticlinal walls; 6. Showing an outer peristomial layer divided by periclinal walls; 7. Division of cells in an inner peristomial layer by anticlinal walls; 8, 9. Continuation of divisions in outer amphithecial layer and endothecium.

ding to the inner peristomial layer, increase to about 24 in number by the formation of anticlinal walls and undergo no further divisions (Figs. 8, 9). Thus, in cross section each of the eight groups comprises three cells of the inner peristomial layer lving opposite two cells of the outer one and develops into two peristome teeth (Figs. 9–12). In longitudinal section the rows of cells of both outer and inner peristomial layers taking part in the formation of the peristomes number from thirty-seven to forty rows. In the view of the longitudinal sections of the upper regions of young capsule, showing the successive stage of development of the teeth, the number of cells in the full length of peristome amounts from twenty to twenty-five (Fig. 14).

Deposition of the peristomial thickenings

As above described, the peristomial layer is divided into eight groups, in which the three rows of the inner peristomial layer correspond with the two rows of the outer layer. And a group gives rise to two teeth of the peristome and the same process is produced in each group. The deposition of the peristomial thickenings takes part on the outer periclinal walls of the cells circumscribed to the endothecium
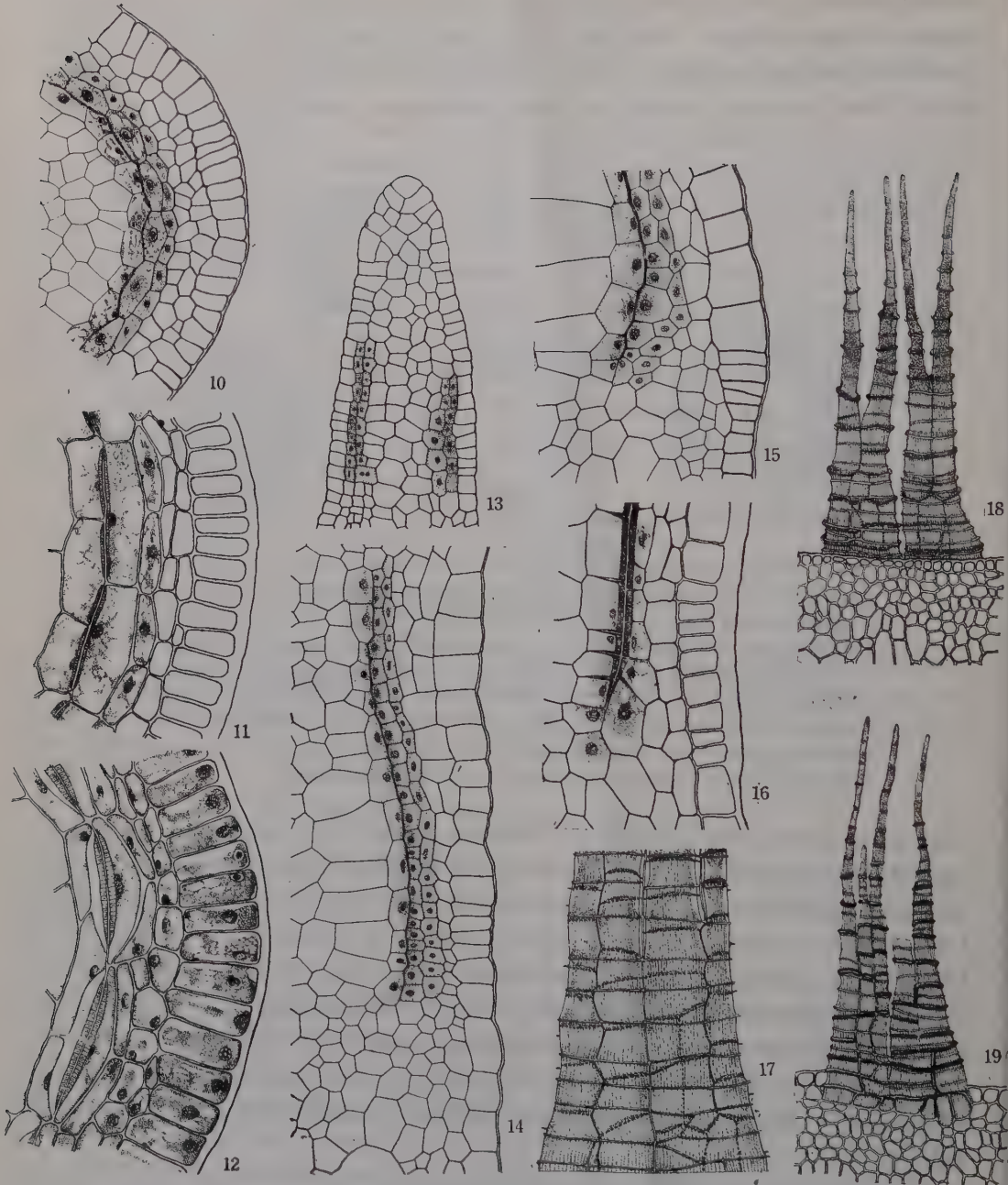
Figs. 10–12.　Cross sections through the amphithecial tissues of older capsules stippled ×300:　10. Showing the peristomial layers in the region of the teeth, just before the deposition of the thickenings;　11, 12. Early stage in the development of the thickening with the adhesion and disappearance of cytoplasm and nuclei.

Figs. 13-19.　Radial sections in the region of the teeth, showing the peristomial thickenings in their successive stage of development stippled, ×300, and teeth of young and old capsules:　13, 14. Showing total length of peristomial layer in an early stage;　15, 16. Showing their thickening in the regions of the basal portion of peristomes;　17. Tangential section of basal portion of the young peristome, ×200;　18, 19. Fully developed teeth, ×110 (18, seen from outside;　19, inside).

(Figs. 10–12).   As the capsules swell, the regions of their opercula become distinguished from the capsules.   Just before the thickenings are deposited, the peristomial cells taking part in thickening lead to the swelling of their nuclei and usually provide with vacuoles (Figs. 10, 11).   And then the cytoplasm and nuclei in the peristomial cells are adhered to the part of the thickenings (Figs. 10, 11, 12, 14, 15, 16).

In succession with the deposition of the peristomial thickenings, the cytoplasm coheres to the part of the thickenings and the vacuoles expand, but the disappearance of the both cytoplasm and nucleus is followed (Fig. 16). After the adhesion and the disappearance of the cytoplasm and nuclei throughout the peristomial thickenings, the initial thickening begins in the basal portion of the peristome and thickening essentially undergoes in the same process throughout the branches of the peristomes (Figs. 15, 16).   The lamellae of the inner side of peristome teeth are well developed, especially they are more pronounced in the basal part of the same.   There are also seen a few longitudinal ridges between each lamella.   In other words the peristomial thickenings continue with the thickenings on the inner wall and give rise to the transverse ridges of the mature peristomes.   In the young stage the transverse and longitudinal thickenings are seen in tangential section, and the same appearance is more striking in an older tooth (Figs. 17–19).

In a word the thickenings extend almost over the inner periclinal walls and on the radial and transverse walls extend as ridge.   Soon after the thickenings have been deposited uopn the teeth, the peristomial cells dry up, the thin parts of the walls shrivel away and disappear, and the teeth become free.

Considerations

In the Haplolepideae the cell divisions of the young sporophyte proceed regularly, and the pattern of the cell divisions is the same with that of *Mnium*, *Funaria* and *Bartramia*, each of which is member of Diplolepideae.

In the amphithecial tissue of the young capsule, the two concentric layers of cells are produced from sixteen cells of the outer peristomial layer and thirty-two cells of the inner layer are seen in each species above said.   In *Ceratodon purpureus* the inner peristomial layer is composed of twenty-four rows of cells, while in *Dicranum japonicum* it is composed from twenty to twenty-four rows of cells, yet the inner peristomial layer is formed by the unequal cells in both shape and size as seen in *Ceratodon purpureus*.   Such a feature was not seen in *Mnium microphyllum*[9] and *Bartramia crispata*.[10]   Toward the base of the branches of teeth the transverse ridges grow wider and wider until finally some of them coalesce with each other in *Ceratodon purpureus*, while in *Dicranum japonicum* the transverse ridges get nearer each other and increase their stripes in number.   The deposition of the thickenings in the peristomial layer is equivalent on the outer and inner

sides of the middle lamellae. In the Diplolepideae the transverse ridges adhere to the inner wall of the peristomial layer, but not to the outer wall as is the case of the Diplolepideae. In *Dicranum japonicum* the vestiges of the radial walls form a zigzag longitudinal line on the inner surface of the tooth, but not on the outside as in Diplolepideae. Of course this line is much shorter than that in *Mnium microphyllum* and *Bartramia crispata*, in which the teeth are not divided into branches. The single peristome of *Dicranum japonicum* is homologous with the inner peristome of the Diplolepideae.

Summary

1. The amphithecium and the endothecium are derived from the cell of quadrant, and the amphithecium is divided further into the inner and outer peristomial layers by periclinal walls.

2. The inner peristomial layer arises from the inner amphithecial layer, undergoing cell division until it is composed of 20—24 cells in cross section by the formation of anticlinal walls.

3. The outer peristomial layer comes to have sixteen cells in cross section by the formation of anticlinal walls. These sixteen cells of the layer undergo no further division.

4. Both peristomial layers form eight groups, each of which is composed of 2 rows of the outer layer and 2 or 3 (mostly 3) rows of the inner layer, and gives rise to two teeth.

5. The ridges of thickening, corresponding to the peristome are laid upon the periclinal walls between the two (inner and outer) peristomial layers.

6. Before the thickenings are deposited, the nuclei in the cells of two layers taking part in the formation of the teeth, are enlarged. And then the protoplasm of both inner and outer peristomial cells moves towards the outer walls of the inner peristomial cells. Following the thickenings the vacuoles appear in cell cavity and both the cytoplasm and the nuclei gradually shrivel away and disappear.

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Taxonomic Study of Cyperaceae V[**]

by Tetsuo Koyama[*]

小山鉄夫: カヤツリグサ科の分類学的研究 5

§ 12. A new classification of the genus *Cladium* in a wide meaning.[***]

The purpose of this article is to divide the genus *Cladium* (sensu lato) into two natural groups which I have recently recognized to be very distinct from each other by their morphological characters including the pollen grains. The genus *Cladium* in a wide meaning consisting of nearly fifty species distributed in the tropical, subtropical and warmer regions of the both hemispheres, is not a very homogeneous group of the family *Cyperaceae* just as the genus *Scirpus*. Because of its heterogeneousness, up till now, under the category of *Cladium* sensu lato were created several similar group, *Machaerina*, *Baumea*, *Vincentia*, *Chapelliera* etc., which were chiefly based upon their external appearances especially on inflorescences and leaves. Some taxonomists including Böckeler, Palla, Stapf and Nees, who created or referred to them, treated all these groups as different genera respectively. Almost all the specialists concerned are, however, of opinion that these groups are attributed to the infrageneric status of the genus *Cladium* sensu lato; for example, Bentham and Hooker in their Genera plantarum, Vol. 3, regarded *Baumea* and *Vincentia* as sections, while C. B. Clarke placed *Baumea*, *Vincentia* and *Machaerina* as the subgenera under the genus *Cladium*.

In the Japanese floristic region including the Ryukyus and the Bonin Islands occur five species of these groups, one in *Cladium* (sensu stricto), three in *Baumea* and one in *Machaerina*. According to my observations on these five species and a considerable number of the tropical species, *Cladium jamaicense*, *Cladium chinense* and *Cladium Mariscus*, members of *Cladium* sensu stricto, are quite different from the other species belonging to *Baumea*, *Vincentia* and *Machaerina* in a very distinct character which corresponds to some characters in their vegetative parts. Speaking on the Japanese species, *Cladium chinense* has achenes almost drupe-like when living and crowned by an inconspicuous somewhat corky stylebase at apex, 3-ranked leaves dorsiventrally compressed, and the corymbose partial inflorescences,

  * Botanical Institute, Faculty of Science, University of Tokyo.

whereas the others, viz. *Cladium nipponense*, *Cladium brevistigma* and *Cladium boninsimae* have hard achenes terminated by a distinct usually scabrous beak, spike-like or paniculate partial inflorescences and 2-ranked leaves terete or bilaterally compressed and sometimes reduced into bladeless sheaths only. The former characters are only seen in *Cladium* in a broad meaning, while *Baumea*, *Vincentia* and *Macha-erina* fall under the latter category. Furhter, an interesting matter is recently offered me by Miss M. Ikuse who is studying on pollen grains and examined those of the three Japanese species, *Cladium chinense*, *Cladium nipponense* and *Cladium brevistigma* in detail. According to her investigation, the pollen grains of *Cladium* (sensu lato) are highly interesting and those of *Baumea* are so peculiar that she reexamined some other samples from different specimens. As seen in Fig. 6, the pollen grains of *Baumea* are of polyforate type, while those ot *Cladium* sensu stricto are of 1-6-aperture type bearing a terminal pore and six sidal ones. Also the differences in somewhat delicate characters of inflorescence and the shape
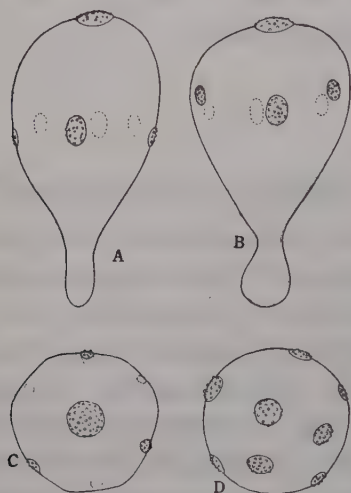
Fig. 6. Pollen grains of *Cladium* sensu lato (offered by Miss Ikuse): A–C. *Cladium chinense* Nees: A & B. lateral views; C. polar view. D. *Machaerina nipponensis* Ohwi et T. Koyama (=*Cladium nipponense* Ohwi) lateral view. × 770.

of achene which are of secondary value in the classification of this group are seen among *Machaerina*, *Baumea* and *Vincentia*, that is to say, *Machaerina* is based upon rather inconspicuously 3-angled achenes usually subabruptly narrowed both at apex and at base and paniculate loose inflorescence often large-sized, *Vincentia* is characterized by comparatively sharply 3-angled achenes gradually narrowed to apex, and gradually attenuate below to a stipelike base, and *Baumea* is separated from *Vincentia* mainly by the cuneate to pyramidal base of achenes sometimes crowned by a depressed stylebase and often densely disposed spikelets.

Thus it seems to be reasonable that the genus *Cladium* in a broad meaning is divided into two independent genera, *Clabium* (sensu stricto) and *Machaerina* including *Baumea* and *Vincentia*. The different characters of these two genera are shown in the table below.

Cladium	Machaerina, *Vincentia* & *Baumea*
ACHENE: Somewhat drupelike, the stylebase almost inconspicuous, corky, smooth.	Hard, 3-angled, the apex crowned by a distinct beak usually hispid or scaberulous, not corky.

STAMENS:	2.	3 (very rarely 2, but always?).
PARTIAL INFLORES- CENCES:	Corymbose.	Slender or conical compound panicle.
LEAVES:	3-ranked, the blades dor- siventrally compressed, coriaceous, prominently scabrous on margins.	2-ranked, the blades equifacial, terete or bilaterally compressed, smooth- margined or nearly so, rarely wanting.
POLLEN:	1-6-aperture type.	Polyforate type.
DISTR. AREA:	Warmer to temperate reg.	Tropical to subtropical regions, domi- nant in Oceania.

The genus *Cladium* created in 1756 by P. Brown is appropriate to a group of Twig-rush represented by the left column of the above table in its original sense. The genus *Mariscus* which was effectively published in 1757 based also on the same group of Twig-rush with *Cladium*, therefore, it should be reduced to the synonimy of *Cladium*. Concerning the generic name of the group represented by the right column of the table, *Vincentia* is frequently adopted because the species belonging to *Vincentia* are a little more than those of *Machaerina*. The genus *Machaerina*, however, was published in precedence of *Baumea* and *Vincentia*, and moreover any of these three genera has neverbeen proposed for 'nomen conservandum'. Accordingly, it may be said that *Machaerina* is legitimate under the present Code of Nomencla- ture, when these three groups are treated as a genus separated from *Cladium* in a narrow sense.

Genus **Cladium** P. Br., Hist, Jamaic.: 114 (1756); Schrad., Fl. Germ. 1: 74 (1806); Kunth, Enum. Plant. 2: 303 (1837); Steudel, Synops. Plant. Glumac. 2: 152 (1855); Böckeler in Linnaea 38: 231 (1874); Benth. et Hook. fil., Gen. Plant. 3: 1065 (1883), pro pte.; Engler et Plantl, Nat. Pflanzenfam. 2-2: 116 (1889), ex pte.; C. B. Clarke in Kew Bull. add. ser. 8: 124 (1908), ex pte.; Ohwi in Mem. Coll. Sci. Kyoto Imper. Univers. ser. B, 18, no. 1: 10 (1943), pro pte. Species typica: *Cladium jamaicense* Cranz.

Cladium chinense Nees, *Cladium Mariscus* R. Br., *Cladium jamaicense* Cranz, *Cladium leptostachyum* Nees et Meyen, *Cladium mariscoides* Torrey.

Genus **Machaerina** Vahl, Enum. Plant. 2: 228 (1806); Kunth, Enum. Plant. 2: 313 (1837); Böckeler in Linnaea 38: 251 (1874), emend. T. Koyama.

Genus *Cladium* (non P. Br.): Benth. et Hook. f. l. c., pro pte.; Engl. et Plantl c., pro pte.; C. B. Clarke l. c., pro pte.; Ohwi l. c., pro pte.

Perennis elata vel mediocris saepe stolonifera, culmo obscure trigono subnudo basi foliis vaginisve obsito, foliis angustis teretibus vel a latere compressis interdum elaminatis, ligulis haud evolutis, inflorescentia paniculata composita decompositave et aut densa aut laxa, paniculis partialibus spicato- vel vere paniculatis sed non corymbosis multispiculatis, spiculis paucisquamatis, nuce trigona coriacea glabra apice rostro coronata, rostro conicolanceolato vel interdum subdepresso semper plus

minus hispidoscabro, stigmatibus 3, perigonii phyllis in setas breves scabras reductis vel nullis, antheris plerumque 3.   Caeteroquin sicut *Cladium* sensu stricto.   Species typica: *Machaerina restioides* Vahl.

Sect. **Machaerina** (Vahl)——Genus *Cladium* aubgen. *Machaerina* (Vahl) C. B. Clarke l. c. 124 (1908).

Sect. **Vincentia** (Gaudich.) T. Koyama, comb. nov.——Genus *Vincentia* Gaudich., Freyc. Voy. Bot. 416 (1826); Kunth l. c. 314; Steudel l. c. 156; Böckeler l. c. 247—— Genus *Cladium* sectio *Vincentia* (Gaudich.) Benth. et Hook. f., Gen. Plant. 3: 1066 (1883).

Sect. **Baumea** (Gaudich.) T. Koyama, comb. nov.——Genus *Baumea* Gaudich., Freyc. Voy. Bot. 417 (1826); Thw., Enum. Pl. Zeyl. 353 (1864); Böckeler l. c. 237 (1874)——Genus *Chapelliera* Nees in Linnaea 9: 417 (1834) ——Genus *Cladium* subgen. *Baumea* (Gaudich.) C. B. Clarke l. c. 124——Genus *Cladium* sectio *Baumea* (Gaudich.) Benth. et Hook. f., l. c. 3: 1065 (1943); Ohwi l. c. 10 (1943).

1. **Machaerina angustifolia** (Gaudich.) T. Koyama, comb. nova
    Vincentia angustifolia Gaudich. Freyc. Voy. Bot. 417 (1826).

2. **Machaerina arfakensis** (Rendle) T. Koyama, comb. nova
    Cladium arfakense Rendle in L. S. Gibbs, Phytogeogr. & Flor. Arfak. Mts. 90 (1917).

3. **Machaerina aromatica** (Merrill) T. Koyama, comb. nova
    Cladium aromaticum Merrill in Philip. Journ. Sci. 9: 59 (1914).

4. **Machaerina arthrophylla** (Nees) T. Koyama, comb. nova
    Chapelliera arthrophylla Nees in Lehm. Plant. Preiss. 2: 77 (1846)——*Cladium arthrophyllum* (Nees) F. V. Müller, Fragm. Phytogr. Austral. 9: 14 (1875).

5. **Machaerina articulata** (R. Br.) T. Koyama, comb. nova
    Cladium articulatum R. Br. Pradr. Fl Nov. Holl. 237 (1810).

6. **Machaerina aspericaulis** (Kükenth.) T. Koyama, comb. nova
    Cladium aspericaule Kükenth. in Bull. Jard. Bot. Buitenz. 3e sér., 16: 309 (1940).

7 **Machaerina australis** (Rich.) T. Koyama, comb. nova
    Vauthiera australis Richard, Ess. Flor. N. Zél. 107, t. 20 (1832)——*Cladium Vauthiera* C. B. Clarke ex Cheeseman, Man. N. Z. Flor. 788 (1906).

8. **Machaerina Bidwellii** (Stapf) T. Koyama, comb. nova
    Vincentia Bidwellii Stapf in Dept. Marine Biol. Carnegie Inst. Wash. 20: 111 (1924), in obs.

9. **Machaerina boninsimae** (Nakai) T. Koyama, comb. nova
    Cladium boninsimae Nakai in Bot. Mag. Tokyo 25: 223 (1911).

10. **Machaerina borneensis** (C. B. Clarke ex Stapf) T. Koyama, comb. nova
    Cladium borneense C. B. Clarke ex Stapf in Trans. Linn. Soc. Bot. 4: 245 (1894).

11. **Machaerina brevipaniculata** (Kükenth.) T. Koyama, comb. nova

Cladium brevipaniculatum Kükenth, in Fedde, Repert. 51: 176 (1942).

12. **Machaerina brevistigma** (Nakai ex Tuyama) T. Koyama, comb. nova
Cladium brevistigma Nakai ex Tuyama in Bot. Mag. Tokyo 49: 509 (1935).

13. **Machaerina complanata** (Bergg.) T. Koyama, comb. nova
Cladium complanatum Bergg. in Minnesks. Fisiog. Sallks. Lund. n. 8: 23 (1877).

14. **Machaerina capillacea** (Hook. fil.) T. Koyama, comb. nova
Chaetospora capillacea Hook. fil., Flor. Tasman. 2: 81, t. 141, A (1858), non Nees.

15. **Machaerina crassa** (Thw.) T. Koyama, comb. nova
Baumea crassa Thwait., Enum. Plant. Zeyl. 353 (1864).

16. **Machaerina crinita** (Stapf) T. Koyama, comb. nova
Vincentia crinita Stapf in Philip. Journ. Sci. 19: 65 (1921).

17. **Machaerina cubensis** (Kükenth.) T. Koyama, comb. nova
Cladiam cubense Kükenth. in Fedde, Repert. 23: 213 (1926).

18. **Machaerina cyperoides** (Merrill) T. Koyama, comb. nova
Cladium cyperoides Merrill in Philip. Journ. Sci. 7: 74 (1912).

19. **Machaerina Deplanchei** (Böcklr.) T. Koyama, comb. nova
Baumea Deplanchei Böckeler in Flora 61: 143 (1878).

20. **Machaerina dissoluta** (Stapf ex Setchell) T. Koyama, comb. nova
Vincentia dissoluta Stapf ex Setchell in Dept. Marine Biol. Carnegie Inst. Washingt. 20: 111 (1924).

21. **Machaerina disticha** (C. B. Clarke) T. Koyama, comb. nova
Cladium distichum C. B. Clarke in Philip. Journ. Sci. 2: 102 (1907).

22. **Machaerina Ekmanii** (Kükenth.) T. Koyama, comb. nova
Cladium Ekmanii Kükenth. in Fedde, Repert. 23: 213 (1926).

23. **Machaerina ensifolia** (Böklr.) T. Koyama, comb. nova
Elynanthus ensifolius Böckeler in Linnaea 38: 264 (1874).

24. **Machaerina elynanthoides** (F. v. Müller) T. Koyama, comb. nova
Cladium elynanthoides F. v. Müller, Fragm. Phytogr. Austral. 9: 31 (1875)
——*Elynanthus australis* Nees in Lehm., Pl. Preiss. 2: 79 (1846).

25. **Machaerina ensigera** (Hance) T. Koyama, comb. nova
Cladium ensigerum Hance in Journ. Bot. 23: 80 (1885).

26. **Machaerina ficticia** (Hemsley) T. Koyama, comb. nova
Cladium ficticium Hemsley, Bot. Challenger Voy. 3: 59, t. 60, f. 5-7 (1885), in obs.

27. **Machaerina falcata** (Nees) T. Koyama, comb. nova
Baumea falcata Nees in Hook, Kew Journ. 6: 29 (1854).

28. **Machaerina glomerata** (Gaudich.) T. Koyama, comb. nova
Baumea glomerata Gaudich., Freyc. Voy. Bot. 416, t. 29 (1826).

29. **Machaerina gracilis** (J. M. Black) T. Koyama, comb. nova

Cladium gracile J. M. Black in Trans. & Proc. Roy. Soc. S. Austr. 53 : 261 (1929).

30. **Machaerina Huttoni** (T. Kirk) T. Koyama, comb. nova
   Cladium Huttoni T. Kirk in Trans. N. Z. Inst. 9 : 551 (1877).

31. **Machaerina insularis** (Benth.) T. Koyama, comb. nova
   Cladium insularis Bentham, Flor. Austral. 7 : 403 (1878).

32. **Machaerina iridifolia** (Baker) T. Koyama, comb. nova
   Cladium iridifolium Baker, Flor. Maurit. 424 (1877).

33. **Machaerina iris** (Ohwi) Ohwi et T. Koyama, comb. nova
   Cladium iris Ohwi in Bot. Mag. Tokyo 41 : 208 (1942).

34. **Machaerina juncea** (R. Br.) T. Koyama, comb. nova
   Cladium junceum R. Br., Prodr. Fl. Nov. Holl. 237 (1810).

35. **Machaerina juncoides** (Elmer) T. Koyama, comb. nova
   Cladium juncoides Elmer, Leafl. Philip. Bot. 3 : 854 (1910).

36. **Machaerina latifolia** (Merrill) T. Koyama, comb. nova
   Cladium latifolium Merrill in Philip. Journ. Sci. 2 : 262 (1907).

37. **Machaerina latissima** (F. v. Müller) T. Koyama, comb. nova
   Cladium latissimum F. v. Müller, Fragm. Phytogr. Austr. 9 : 15 (1875).

38. **Machaerina laxa** (Benth.) T. Koyama. comb. nova
   Cladium laxum Bentham, Flor. Austral. 7 : 405 (1878).

39. **Machaerina macrophylla** (Böcklr.) T. Koyama, comb. nova
   Vincentia macrophylla Böckeler, Allg. Bot. Zeitschr. 112 (1896).

40. **Machaerina Maingayi** (C. B. Clarke) T. Koyama, comb. nova
   Cladium Maingayi C. B. Clarke in Hook. f., Flor. Brit. India 6 : 674 (1896).

41. **Machaerina Gaudichaudii** (W. F. Wight) T. Koyama, comb. nova
   Cladium Gaudichaudii W. F. Wight in Contrib. U. S. Nat. Herb. 9 : 230 (1905).

42. **Machaerina Melleri** (Baker) T. Koyama, comb. nova
   Cladium Melleri Baker in Journ. Linn. Soc. 21 : 451 (1885).

43. **Machaerina micranthes** (C. B. Clarke) T. Koyama, comb. nova
   Cladium micranthes C. B. Clarke in Kew Bull. add. ser. 8 : 46 (1908).

44. **Machaerina Milnei** (C. B. Clarke) T. Koyama, comb. nova
   Cladium Milnei C. B. Clarke in Kew Bull. add. ser. 8 : 46 (1908).

45. **Machaerina monticola** (Guillaumin) T. Koyama, comb. nova
   Vincentia monticola Guillaumin in Bull. Mus. Hist. Nat. Paris 2e sér., 9 : 304 (1937).

46. **Machaerina Muelleri** (C. B. Clarke) T. Koyama, comb. nova
   Cladium Muelleri C. B. Clarke in Kew Bull. add. ser. 8 : 47 (1908).

47. **Machaerina monocarpa** (J. M. Black) T. Koyama. comb. nova
   Schoenus monocarpus J. M. Black in Trans. & Proc. Roy. Soc. S. Atstr. 52 : 225 (1928).

48. **Machaerina malesiaca** (Stapf) T. Koyama, comb. nova
    Vincentia malesiaca Stapf in Journ. Linn. Soc. 42: 178 (1914).

49. **Machaerina nipponense** (Ohwi) Ohwi et T. Koyama, comb. nova
    Cladium nipponense Ohwi in Mem. Coll. Sci. Kyoto Imper. Univ. ser. B, 18, no. 1: 11 (1943).

50. **Machaerina nukuhivensis** (Forst) T. Koyama, comb. nova
    Cladium nukuhivense Forst ex Brown in Bull. Bishop. Mus. Honolulu no. 84: 111 (1931).

51. **Machaerina pantopoda** (Baker) T. Koyama, comb. nova
    Cladium pantopodum Baker in Journ. Linn. Soc. 21: 451 (1885).

52. **Machaerina philippinensis** (Merrill) T. Koyama, comb. nova
    Cladium philippinense Merrill in Philip. Journ. Sci. 5: 171 (1910).

53. **Machaerina ponapensis** (Ohwi) Ohwi et T. Koyama, comb. nova
    Cladium ponapense Ohwi in Journ. Jap. Bot. 18: 136 (1942).

54. **Machaerina procera** (S. T. Blake) T. Koyama, comb. nova
    Cladium procerum S. T. Blake in Trans. Roy. Soc. S. Austr. 67: 57 (1943).

55. **Machaerina riparia** (Benth.) T. Koyama, comb. nova
    Cladium riparium Bentham, Flor. Austral. 7: 405 (1878).

56. **Machaerina Robinsonii** (Merrill) T. Koyama, comb. nova
    Vincentia Robinsonii Merrill in Philip. Journ. Sci. 11: 258 (1916).

57. **Machaerina rubiginosa** (Soland ex Forst) T. Koyama, comb. nova
    Schoenus rubiginosus Soland ex Forst, Prodr. 89 (1786)——*Cladium glomerata* R. Br., Prodr. Fl. Nov. Holl. 237 (1810).

58. **Machaerina punctata** (R. Br.) T. Koyama, comb. nova
    Schoenus punctatus R. Br. Prodr. Fl. Nov. Holl. 232 (1810)——*Schoenus nudus* Steudel, Syn. Pl. Glumac. 2: 165 (1855).

59. **Machaerina samoensis** (C. B. Clarke ex Stapf) T. Koyama, comb. nova
    Cladium samoense C. B. Clarke ex Stapf in Trans. Linn. Soc. Bot. 4: 245 (1894).

60. **Machaerina schoenoides** (R. Br.) T. Koyama, comb. nova
    Cladium schoenoides R. Br. Prodr. Fl. Nov. Holl. 237 (1810).

61. **Machaerina scirpoidea** (Benth. et Hook. f. ex Hemsl.) T. Koyama, comb. nova
    Cladium scirpoideum Benth. et Hook. f. ex Hemsley, Bot. Challenger Voy. 3: 59. f. 1–4 (1885).

62. **Machaerina Sinclairii** (Hook. fil.) T. Koyama, comb. nova
    Cladium Sinclairii Hook. fil., Handb. N. Zeal. Fl. 304 (1864).

63. **Machaeria pulchra** (Ridley) T. Koyama, comb. nova
    Cladium pulchrum Ridley in Journ. Federated Mal. States Mus. 6: 192 (1925).

64. **Machaerina sinuata** (Ridley) T. Koyama, comb. nova
    Cladium sinuatum Ridley in Trans. Linn. Soc. Bot. 9: 243 (1916).

65. **Machaerina stradbrokensis** (Domin) T. Koyama, comb. nova

Cladium stradbrokense Domin in Bibliothec. Bot. 85 : 476 (1915).

66. **Machaerina sucinonux** T. Koyama, spec. nova e sectione *Machaerina* (Vahl).

Perennis. Culmus erectus 5–9 dm altus firmus a latere compressus stramineo-virens opacus praeter angulos scaberulos laevis striatus infra medium foliatus 3–4-nodosus, internodiis 10–17 cm longis vaginis foliorum fere obsitis.　Folia caulina erecta quam inflorescentia paullo breviora vel aequantia, laminis ensiformibus a latere valde compressis 20–45 cm longis 4–5.5 mm latis subcoriaceis glaucoviridibus in sicco laevibus interdum margine sursum scaberulis apice abrupte contractis breviter subacutis basi in vaginas longas culmum subarcte circumdantes compressas vix attenuantibus, ligulis vix productis.　Inflorescentia paniculata bis ter composita elata sed sublaxa 40–55 cm longa 3–5 cm lata ; paniculae secundariae plures 10–25 cm longae ex unica bractea 1–3-nae contiguae longe exserte vel subincluse pedunculatae valde ramosae, pedunculis subcompresse triquetris laevibus, bracteolis spathaceis longiuscule vaginantibus, paniculis tertiariis linearibus sublaxe plurispiculosis ; Bracteae infetiores foliaceae paniculis partialibus suis breviores basi longe vaginatae, superiores in vaginam sursum ampliatam reductae.　Spiculae maturitate ovoideae ellipsiodeaeve 3–4 mm longae 1.5–2 mm in diametro fuscae 3–4-squamatae uniflorae. Squamae imbricatim dispositae ovatoellipticae vel ellipticae membranaceae fusce-scentes et fuscostriatae naviculares sursum hispidoscabrae spice subabrupte angustatae acutae mucronatae, carina uninervata ; summa vacua 2.5 mm longa ; sequens fertilis summa paullo grandior ; inferiores etiam vacuae eae superiores 2 similes sed paullo angustiores brevioresque dorso fere ex toto hispidae.　Nux ellipsoidea 2–2.2 mm longa 1.5 mm in diametro obtuse trigona facie fulvosucina nitida grosse rugosa basi sub-sessilis ; stylopodium rostriforme conicolineare cum 1/2-nuce subaequans ex toto perdense tomentosohispidum albescens ; stylus gracilis longus erectus, stigmatibus 3 recurvis longiusculis papulosis.　Stamina 3.

Bonin : Ins. Hahajima (K. Sawatari, n. 86 !—Holotypus in Herb. Univ. Tokyo-ensis).

67. **Machaerina tenax** (Hook. fil.) T. Koyama, comb. nova
    Lamprocarya tenax Hook. fil., Flor. N. Zeal. 1 : 277 (1853).
68. **Machaerina teretifolia** (R. Br.) T. Koyama, comb. nova
    Cladium teretifolium R. Br. Prodr. Fl. Nov. Holl. 237 (1810).
69. **Machaerina tetragona** (R. Br.) T. Koyama, comb. nova
    Lepidosperma tetragonum R. Br., Prodr. 235 (1810).
70. **Machaerina undulata** (Thw.) T. Koyama, comb. nova
    Cladium undulatum Thwaites, Enum. Plant. Zeyl. 353 (1864).
71. **Machaerina vaginalis** (Benth.) T. Koyama, comb. nova
    Cladium vaginale Bentham in Benth. et Müller, Flor. Austral. 7 : 408 (1878).

Summary

1. In this article, the author intended to divide the Genus *Cladium* in a wide meaning into two natural groups, *Cladium* sensu stricto and *Machaerina*, by their morphological characters including the pollen grains.

2. In the former group, leaves are coriaceous, dorsiventrally flattened and always 3-ranked, and achenes are somewhat drupe-like and not crowned by distinct beak at apex, whereas the latter has bilaterally compressed or terete leaves usually 2-ranked and has achenes crowned by a distinct beak often scabrous at tip.

3. This division also very well agrees with the features of pollen grains, which are represented by 1–6-aperturate type in the former and polyforate type in the latter.

4. Concerning the generic name of the latter, Vahl's *Machaerina* is legitimate.

Studies on the Adaptation of Yeast to Copper XII.
Amino Acid Synthesis as a Copper Resistance
Mechanism of a Variant.[*]

by Yutaka Arakatsu[**] and Joji Ashida[***]

荒勝　豊・芦田讓治：　酵母の銅に対する適応的変異現象の研究 XII.
　　　　　　銅耐性変異株の耐性機序としてのアミノ酸合成能

In the preceding paper[1] culture conditions which favoured the growth and the brown pigmentation of *Saccharomyces ellipsoideus* in copper-containing media were reported, with the conclusion that the predominating copper-resistant substrain, R_{1b}, can synthesize glutamic acid and aspartic acid from ammonium sulfate even in copper media in which the parent strain cannot. Nitrogen metabolism seems to be important when one considers how yeast cells are injured by copper and how resistant cells can survive the copper injury. Hence some experiments have been made to see the effect of various amino acids on the inhibition of sensitive cells by copper. Here the results are reported.

Material and Method

The strain of *Saccharomyces elliposideus* used was the same one as in the previous reports[1,2,3,4,5]. The stock cluture of the parent strain was kept on malt extract agar, and cells were cultured on MH* -agar before each experiment. The copper resistant substrain, R_{1b}, was obtained, as before, by streaking the parent strain on the MH-slant containing $1 mM$ of $CuSO_4$, followed by successive subcultures on the same medium. The growth was brown when the culture medium contained certain concentrations of copper[1,6]. R_{1b} cultured on the copper free MH-agar is called R_{1b} (0).

For experimental cultures, glycine in the h-medium** was replaced by each one of other 19 amino acids or by ammonium sulphate, to equal the amount of nitrogen.

Commercial noodle-shaped agar was added to 2% for slants, and to 1.5% for pour-plating media. The sterilization was made under 10 psi. Copper media were prepared by mixing a calculated amount of sterile solution of recrystalized $CuSO_4 \cdot 5H_2O$ with the sterilized culture media before solidification, at 43–45° C. The incubation temperature was 30° C.

The effect of nitrogen source in the case of the parent strain surviving the copper injury was determined in the two ways: a) by determining the " variation point ", namely the minimum copper concentration at which the growth can be resumed only by secondarily developing resistant colonies, and b) by determining the survival ratios at various copper concentrations.

The first method is based on a hypothesis that cells of the inoculated sensitive strain will not be overgrown on a copper medium by resistant variant cells, so far as the former can grow rapidly enough thanks to the supplemented amino acid. Graded amounts of copper were added to each of the media provided with different nitrogen sources. A loopful of a cells suspension (*ca.* 10^8 cells/ml.) was streaked straight on each slant, and the growth behaviour was observed after a week's incubation at 30° C.

For the second method, a definite volume of cell suspension was mixed with each of different media at 43° C, before they solidified. Portions of each mixture were mixed with measured volumes of a copper solution, and 10 or 20 ml each of them was poured into a Petri dish severally, the control plates containing no added copper. The ratio of the number of visible colonies appearing in the copper plate to that in the corresponding control plate was named the survival ratio.

The carbon dioxide evolution was measured with Warburg's manometer under the nitrogen atmosphere at 30° C.

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* KH_2PO_4 3g, $MgSO_4 \cdot 7H_2O$ 2g, peptone (Funai) 5g. cane sugar 100g, distilled water 1,000 ml, malt extract (Bé 8%) 360 ml.

** KH_2PO_4 3g, $MgSO_4$. $7H_2O$ 1g, $(NH_4)_2SO_4$ 1g, glycine 1g, cane sugar 50g, distilled water 1,000 ml, thiamine 200μg, pyridoxine 200μg, nicotinic acid 200μg, pantothenic acid 200μg, biotin 2μg, inositol 10,000μg, riboflavine 200μg, PABA 200μg.

Results

Variation point. The parent strain was streaked on the 20 kinds of media differ-ing from each other in the nitrogen source. The growth patterns observed after 7 days of incubation are summarized in Table 1. This shows that some amino acids enabled the streaked cells grow rather uniformly even when the copper concentra-tion of the medium was high, while some others did not even with lower concentra-tions of copper. In the latter case, the growth stopped when a thin film of cells was formed, followed by the growth of variant colonies distributed spottedly on the background film. The growth pattern and the colour tone of variant colonies were not quite the same for all kinds of the medium; in the extreme cases, for example,

Table 1. Variation points of the parent strain streaked on media containing different amino acids and graded amounts of copper, observed after 7 days of incubation. $+$: growth by secondary colonies. $-$: growth without secondary colonies.

Class	Nitrogen source	Cu concentration, 0.1 mM/1								
		2	3	4	5	6	7	9	10	13
E	NH$_4$SO$_4$ only	$+$								
	1-Histidine-Hcl	$+$								
	1-Arginine-Hcl	$+$								
	1-Lysine-Hcl	$+$								
D	Tyrosine	$\pm$	$+$							
	dl-β-Phenylalanine	$-$	$+$							
	1- Proline	$-$	$+$							
C	dl- Valine	$-$	$\pm$	$+$						
	Glycine	$-$	$-$	$+$						
	dl-α-Alanine	$-$	$-$	$+$						
	dl-α-Aminoisobutylic acid	$-$	$-$	$+$						
B	dl- Methionine	$-$	$-$	$\pm$	$+$					
	1- Leucine	$-$	$-$	$-$	$+$					
	dl- Isoleucine	$-$	$-$	$-$	$+$					
	dl- Serine	$-$	$-$	$-$	$+$					
	dl-α-Aminobutylic acid	$-$	$-$	$-$	$-$	$+$				
	dl- Threonine	$-$	$-$	$-$	$-$	$\pm$	$+$			
A	1- Na-Glutamate	$-$	$-$	$-$	$-$	$-$	$-$	$\pm$	$+$	
	1- Na-aspartate	$-$	$-$	$-$	$-$	$-$	$-$	$-$	$-$	$+$
	dl- Norleucine	$-$	$-$	$-$	$-$	$-$	$-$	$-$	$-$	$+$

the primary growth was thicker on the medium containing phenylalanine than on others, and the secondarily growing colonies were white on the one containing methionine. However, the two-stepped growth observed on any of the tested media seemed to fall in the same category of phenomena as that observed on the MH-medium containing copper[3,4,6].

The results as shown in Table 1 were reproduced in the general trend, in repeated experiments. And the amino acids tested were classified from A to E

according to their ability of helping the smooth growth of the sensitive strain in the presence of copper.

Survival ratio. The survival ratios of the parent strain were determined by the pour-plate method, using the media containing each of amino acids belonging to classes A, B, C and D according to the foregoing experiment. The results, as represented in Table 2, show that some of the amino acids of each class do, and the others do not, give significant survival values at copper concentrations corresponding to the variation point of the class. Noteworthy is the fact that, with the copper medium containing glutamate or aspartate, even the parent strain can survive as

Table 2. Survival ratios of the parent strain in the media containing one of the various amino acids and different concentrations of copper, compared with those of R$_{1b}$ (0) in the medium containing glycine.

Strain	Class of amino acid	Amino acid	Cu concentration, 0.1 mM/1									
			3	3.5	4	4.5	5	6	8	10	12	15
Parent	D	Phenylalanine	52	45	14	8	0					
		Proline	35	—	0	0						
	C	Valine	45	—	23	—	0					
		Glycine*	65	20	9	0						
		α-alanine	14	—	3	0						
	B	Leucine	35	17	0							
		Isoleucine	28	9	0							
		Serine			53	—	29	0				
		Aminobutyric acid				9	9	0				
		Threonine				50	32	0				
	A	Na-glutamate						75	50	32	18	5
		Na-aspartate						90	22	0		
		Norleucine				0	0	0	0			
R$_{1b}$ (0)	C	Glycine*	100	100	100	100	81	54	0	0		

* Standard medium.

well as, or even better than, the resistant strain plated with the standard copper medium of which the organic nitrogen source was glycine.

It is suspected that these dicarboxylic amino acids catch more copper outside cells than other amino acids do. It was reported in the previous paper[1] that the parent strain grown on the copper medium containing glutamate coloured brown conspicuously, indicating that the metabolism was interfered with by copper which had entered in the cell. In order further to make matter clear, the survival ratios of the parent and the resistant strains were compared to each other using the media containing glycine, glutamate and aspartate. The results are presented in Table 3. With the glycine medium, the survival of the parent strain became nil at the copper concentration where the survival of the resistant strain was 95%. The

Table 3.　Comparisom of survival ratios of the parent and of R_{1b} (0) strains.

Amino acid in medium	Strain	Cu concentration, mM/1										
		0.3	0.35	0.4	0.45	05	0.6	0.7	0.8	1.0	1.2	1.5
Glycine	Parent	65	20	9	0	0	0	0				
	R_{1b} (0)	100	100	100	95	81	54	17	0			
	Ratio P : R_{1b} (0)	0.65	0.20	0.09	0	0	0	0				
Aspartate	Parent							90	22	0	0	0
	R_{1b} (0)							100	100	95	80	78
	Ratio P : R_{1b} (0)							0.90	0.22	0	0	0
Glutamate	Parent							75	50	32	18	5
	R_{1b} (0)							85	70	55	40	30
	Ratio P : R_{1b} (0)							0.89	0.71	0.58	0.45	0.17

circumstances were the same also with aspartate, although the copper concentration at which practically no parent cells survived was higher in this case. With glutamate, on the other hand, the survival ratio of the parent strain was not much inferior to that of the resistant strain up to high copper concentrations. If glutamate were effective only by reducing the effective concentration of copper, the resistant strain should keep very high survival values so long as the survival ratio of the parent strain is more than a few percent. Hence it is concluded that if the medium contains a certain amount of glutamate, the parent strain can survive well in high concentrations of copper, not solely because the effective copper concentration is reduced by glutamic acid, but because this amino acid helps the grownh of the parent cells in the presence of copper. According to polarographic determinations, too, in the glutamate medium to which $CuSO_4$ was added to give concentrations of 0.6 ·and 1.2 mM/1 the activity of copper ion was roughly the same as in the standard and the ammonium sulphate media to which the same amounts of copper were added respectively.

　　Hence it can be surmised that, in the presence of copper above a certain level, the parent strain cannot synthesize a sufficient amount of glutamic acid (or intermediates to be derived from this) from ammonium and glycine, as well as other amino acids so far tested.

　　All the R_{1b} (0) cells can grow in the standard medium containing 0.45 mM of copper (Table 2). This may mean that this strain can undergo the nitrogen metabolism necessary for growth well up to this concentration, using glycine and ammonium as the nitrogen sources. The decline of survival ratio ot R_{1b} (0) due to a further increase in copper concentration can be alleviated if glycine is replaced by glutamate. Hence even in R_{1b} (0) the glutamic acid synthesis from glycine and ammonium seems to limit the growth at the high copper concentrations.

　　A remarkable difference between the parent and R_{1b} (0) strains seems to be involved in the ability of synthesizing glutamic acid from simple nitrogen sources

within a certain range of copper concentration. And there is a possibility that the copper resistance of $R_{1b}(0)$ involves as an important mechanism a relative immunity of glutamic acid synthesis from inhibition by copper.

Aspartate, on thh other hand, does not help the survival of the parent strain so well as glutamate, although it is rather more effective than glutamate for $R_{1b}(0)$ strain. The concentration of copper ion did not seem to be lowered by aspartate according to polarographic determinations. Hence it may be that when aspartate is supplied sufficiently, the nitrogen metabolism necessary for growth does not suffer complete inhibition almost up to 1.0 mM of copper in the case of $R_{1b}(0)$. Aspartate seems to be in a situation different from glutamate as to the copper injury of the particular two strains in question.

CO$_2$ evolution. It is shown by the experiments described in the above and in the preceding paper[1], which have dealt with the growth in several days, that glutamate and aspartate are effective in reducing the copper injury. In order to see if these amino acids reveal their effectiveness within a short time of action, copper inhibition of the anaerobic evolution of CO_2 was determined replacing glycine in the standard medium by glutamate or aspartate.

Representative sets of experimental results are illustrated in Figs. 1 and 2. Since the amount of cells used for measurements was not determined by weight, comparisons are possible only among the values in each set of experiments.

The first set, represented in Fig. 1, shows that the CO_2-evolution of the parent strain (c) is, and that of $R_{1b}(0)$ (b) is not, inhibited severely by 0.6 mM of copper. When, however, glycine in the medium was replaced by glutamate (g, Fig. 2) and aspartate (h), the copper inhibition was not so strong. By eliminating sugar in the medium (i) it was shown that the CO_2 measured with other media originated mostly from sugar, and not from amino acids.

Discussion and Conclusion

When the sensitive strain is inoculated on the synthetic h-medium which contains 0.4 mM or more of CuSO$_4$, there occurs a slight confluent growth, followed by the growth of discrete secondary colonies consisted of copper resistant variant cells. It is assumed in this case that, if the sensitive cells, being helped by some agent, can grow on the copper medium so well as the resistant variant, they will continue growing on and will not let the latter have an advantage over them. The method of "variation point" is based on this idea. And amino acids were tested by this method how far they can help the sensitive strain growing in the presence of copper. The idea is supported by the following experiment.

The parent strain was inoculated on the medium containing glutamate and 0.8 mM of copper, and three successive subcultures were made on this medium. There virtually appeared no secondary colonies. The copper resistance of the culture was
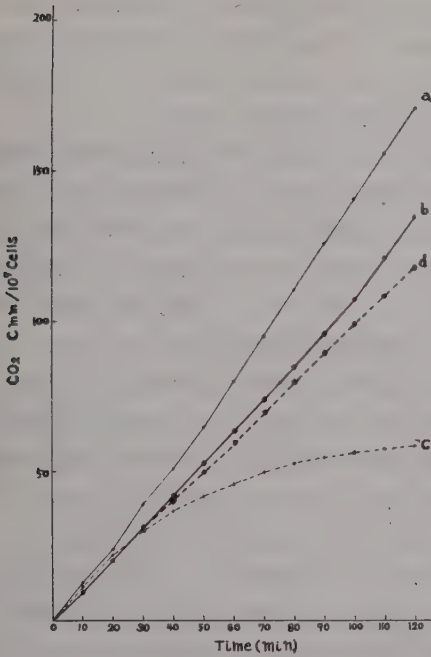
Fig. 1
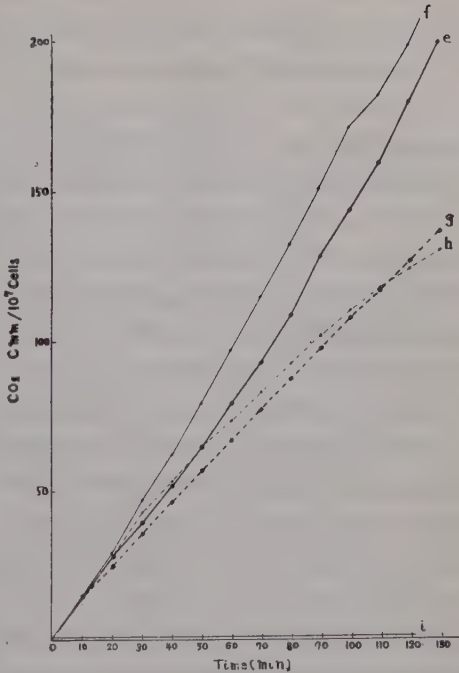
Fig. 2

Figures. The CO_2-evolution under the nitrogen atmosphere by about 10^7 cells suspended in 1.1 ml of the standard culture medium modified as in the following:

Curve	a	b	c	d	e	f	g	h	i
N-Source	s	s	s	s	glut.	asp.	glut.	asp.	glut. or asp.
Sugar	+	+	+	+	+	+	+	+	−
Copper	−	−	+	+	−	−	+	+	−
Strain	P	R	P	R	P	P	P	P	P

N–Source, s: standard (Glycine-$(NH_4)_2SO_4$); glut: glutamate-$(NH_4)_2SO_4$; asp: aspartate-$(NH_4)_2SO_4$

Sugar, −: 50 g/1 sucrose; −: no sugar added.

Copper, −: 0.6 ml/1 $CuSO_4$ (abscissa representing the time after the addition of copper); −: no copper added.

Strain, P: parent strain; R: $R_{1b}(0)$.

then determined by plating in 1 mM Cu-MH-medium, after a passage through the copper-free MH-medium. The culture was found to remain as sensitive as the original strain, notwithstanding that the copper concentration of the subculturing medium was so high as to make resistant cells predominate, if the amino acid in the medium belonged to the classes B, C, D or E in Table 1. Similar experiments were performed by subculturing the sensitive strain on the media which contained, together with various amino acids, copper at the concentrations by from 0.05 to 0.1

mM lower than the variation point characteristic of the respective amino acids. It was always found that the yeast cultured at copper concentrations below the variation point remained unpredominated by resistant variant. The classes of amino acids as shown in Table 1 are expected, therefore, to represent how certain amino acids help the sensitive cells overcoming inibition by copper.

However, when amino acids were tested for their ability of allowing the sensitive strain show significant survival values at higher copper concentrations (Table 2), leucines of B class were found less effective, and phenylanine of D class was more effective than is expected from the results represented in Table 1. The discrepancy according to the method may be due to a difference in what are measured. The survival ratio depends on the number of visible colonies whether these are composed of sensitive cells or of resistant cells produced from them by variation. The variation point is, on the other hand, recorded high when the primary growth of the streaked cells was favoured by an amino acid so well as for the occurrence and the predominance of variant cells to be retarded. Another possible explanation of the discrepancy may be found in the fact that, in the survival ratio method, in which single cells are imbedded in the agar medium separately, a slight difference in the initial growth vigour often results in a marked difference in the number of visible colonies, because a clone consisted of a few cells is apt to lose the chance of further growth in copper media if the growth rate of it is just below a critical level, while it will grow to be a visible colony if the growth rate at this initial stage is just above the level.

At any rate, of the amino acids so far tested, the two, glutamic acid and aspartic acid, are the most effective by either of the two methods. The suspicion that glutamic acid might catch and detoxicate copper ion outside the cell is dispelled by the polarographic determination of copper ion in the medium, and by the comparison of survival ratios of the parent and of the $R_{1b}(0)$ strains in the copper media containing glutamate (Table 3). This table shows that the sensitive parent strain is relieved from copper inhibition by glutamic acid so that it can give survival ratios comparable to those of the resistant $R_{1b}(0)$ strain. It is attractive to suppose that one of the resistance mechanisms of $R_{1b}(0)$ cells is the ability of synthesizing a sufficient amount of glutamic acid from simple nitrogen sources in the presence of copper.

Table 3 shows, on the other hand, that aspartate raises the survival ratio of $R_{1b}(0)$ even higher than glutamate, while reverse is the case for the parent strain. Accoding to polarographic determination, the concentration of copper ion in the aspartate medium was not lower than those containing glutamate, glycine, or ammonium. The brown pigmentation of cells grown on the copper-containing aspartate or glutamate medium, as described in the previous report[1], indicates that a considerable amount of copper has entered the cells. Aspartic acid seems to play a role somewhat different from glutamic acid in the sensitive as well as in R_{1b} cells

growing on copper media.

Manometric measurement of anaerobic CO_2-evolution revealed that glutamate or aspartate could alleviate the inhibition by copper even in a short time of contact with cells.

The above-mentioned effects of amino acids were much less when $(NH_4)_2SO_4$ was omitted from the media. The effect of glutamate and aspartate was, on the other hand, reduced when some other amino acids were supplemented to them. No difinite deduction is possible from these results, since the network of nitrogen metabolism, as its disturbance by copper, must be complicated. There is, however, a possibility that the most crucial difference between the sensitive and $R_{1b}(0)$ strains is in the ability of synthesizing glutamic acid under the influence of copper. Various amino acids other than glutamic acid also can help the sensitive cells grow in media containing the copper at concentrations which may perhaps correspond to those inhibiting the metabolism of the respective amino acids.

Summary

1. The effect of various amino acids alleviating the copper injury of a strain of *S. ellipsoideus* was observed with three methods; a) determination of the highest copper concentration which does not permit a preferential growth of secondary resistant colonies on the streaked parent strain; b) comparison of the survival ratios of the parent strain and of a copper-trained substrain at various copper concentrations; c) reversal of copper inhibition of CO_2 evolution.

2. The inhibition by copper was much reduced by the presence of Na-glutamate or Na-aspartate in the medium.

3. The copper resistance of the predominating resistant strain, R_{1b}, seems to rest on the ability of synthesizing gultamic acid in the presence of copper.

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Studies on the Systematic
Position and Constitution of Pteridophyta.

7) Vascular Behaviours in the Phyllomophore of
Helminthostachys zeylanica Hooker.

by Makoto NISHIDA*

西田　誠：　羊歯植物の分類学的位置と構成に関する研究
7）　ミヤコジマハナワラビの担葉体中における維管束走行

In 1952 the writer reported on the vascular dichotomy in the phyllomophore of *Ophioglossum*. Also in the same year he expanded his discussions to all the genera belonging to *Ophioglossales*.

Concerning the genus *Helminthostachyr*, however, all he could do was to guess the vascular behaviour from works of classic authors, as he had no opportunity to getting access to the real specimens, and as the literatare he could get in touch with was quite limited.

Judging from the drawings of Farmer and Freeman (1899), the vascular branching from the phyllomophore toward the fertile frond (sporophyll) seems to be supplied through the extra-marginal method, as in *Osmundopteris* of *Botrychiaceae* As this plant seems to be much like *Helminthostachys* in many respects including the development of the long phyllomophore, the present writer assumed that the both might resemble each other also in their vascular systems. However, as the descriptions by Farmer and Freeman are not based on serial sections, no definite idea could be given by them about the manner in which the vascular bundles in the basal part of the phyllomophore are connected with those in the upper part. The writer, therefore, has always been thinking it necessary to collect sufficient quantity of the material in question to establish a firmer and more correct concept of the vascular type of *Ophioglossales*. *Helminthostachys zeylanica* is distributed in tropical and subtropical regions, and the northern-most border line of its territories in the Far East passes the central area of the Loochoo (or Ryukyu) Islands: this species is found in Islands Okinawa and Kume, though not abundantly. If one wishes to collect a sufficient lot of specimens he should rather visit Island Miyako or the archipelago of Yaeyama, the islands scattered in the southern-most area of the Loochoos.

* Biological Institute, Chiba University, Konakadai, CHIBA, Japan.
千葉大学文理学部生物学教室

In the fall of 1953 the writer was in the occupied Loochoo Islands for two months, under the auspices of the Japanese Ministry of Education and being permitted by the U. S. Far East Forces, for the purpose of collecting ferns. He could get many of various size, young and mature, specimens of *Helmnthostachys zeyianica*, as the prize of the trip. These specimens were pickled and brought home for closer inspections. Meanwhile, Nozu seems to have obtained some specimens of this species by mail from a plant collector in the Loochoos, and has published a report of his observations (1955) which are detailed as to certain features of the ramification of veins in the phyllomophore. Certainly he has made a little progress beyond Farmer and Freeman. His report, however, seems to lack the observation of the vascular division in the basal part of the phyllomophore. This part must be very important, for the vascular supply toward the sporophyll originates in it. Therefore, it is very hard to suppose otherwise than that his specimens might be incomplete and has lost the rhizome or the lowest part of the phyllomophore. At any rate, the whole-length picture of the vascular system in the phyllomophore has not been given yet.

Having studied thoroughly the specimens brought back with him, the present writer has come to the conclusion which is deviated a little from what he put forward before, and at the same time he has found certain features which should be interpreted in the way different from that of Nozu's.

In the previous papers, the writer assumed that the methods of branching and also of the initiation of the vascular supply for sporophyll, in *Helminthostachys zevlanica*, would be "extra-marginal". This was pointed out by Nozu not to be true. Instead, he concluded that the method should be marginal. The writer now has also fonnd that the method is not the true extra-marginal one, but he would not agree with Nozu whose descriptions seem to be rather vague.

The writer considers the branching method in the phyllomophore of *Helminthostachys zeylanica* to be very unique and proper to the species. No other plants in *Ophioglossales* have ever shown a branching method like such. It seems, however, that the method must be in close relation to the extra-marginal method, probably being its modification.

Material and Methods

All the specimens were collected by the writer himself in Islands Iriomote, Kobama and Miyako, Loochoos, in September and October, 1953.

The specimens were immersed, immediately after collection, in formalin-acetic acid-alcohol and were brought to his laboratory. Serial sections of the phyllomophore, 15 μ thick, were prepared by the ordinary paraffin method and were stained with Heidenhain iron-alum haematoxylin, with or without the counter stain, safranin.

Observations

The vascular trace for the phyllomophore originates from the solenostelic main bundle in the rhizome and it soon becomes mesarch (Figs. 1 and 2). This is first dichotomy which is similar to that found by Chrysler (1945) in *Botrychiaceae*. Nozu (1950, 1955) called such branching in *Sceptridium japonicum* Lyon " sympodial dichotomy ". In *Helminthostachys zeylanica* it seems more natural to call this kind of branching " monopodialized dichotomy ", for the creeping rhizome of this plant produces the phyllomophore only from its upper, dorsal side. Then the second dichotomy occurs in a plane perpendicular to that of the first, and the trace is divided into two equal mesarch strands (Figs. 3 and 4). Soon thereafter the third dicotomy takes place in a plane perpendicular to the previous plane, namely in a plane parallel to that of the first dichomy, and each of two mesarch strands is subdivided into two. Hence there are four strands which have changed the status from mesarch to endarch, as viewed in cross sections (Fig. 5). These features had already been described in detail by classic authors, Farmar and Freeman[3] (1899) and Lang[4] (1915), long before Nozu's report[9] (1955) appeared.

Among these four strands the adaxial ones may give off, from their adaxial ends, the vascular bundles toward the sporophyll. In smaller or younger plants, both of the two adaxial strands play this role of contributing to the sporophyll, but in larger plants only one of them will take part in this division (Fig. 6). The method as such could by no means be called irregular or provisional as it has been expressed by Nozu (1955)[9]. It must rather be characteristic of *Helminthostachys*.

Before the vascular supply for the sporophyll departs from the phyllomophore, these four strands divide themselves, increase in number to eight or up to twelve in total, and will be arranged to take C- or ∩-shaped pattern in cross sections (Figs. 6—8). Meanwhile, either the right or the left adaxial end of the ∩-like arrangement of strands begins to behave very peculiarly, as is shown in Figs. 9—11 : for example, a strand is given off from the right edge of the end-standing strand in the adaxial left side of the ∩-like arrangement, and this strand again separates successively off another branch-strand which then makes a spiral and anticlockwise involution, goes into the pith, and turns itself inside-out until its xylem is faced to the axis
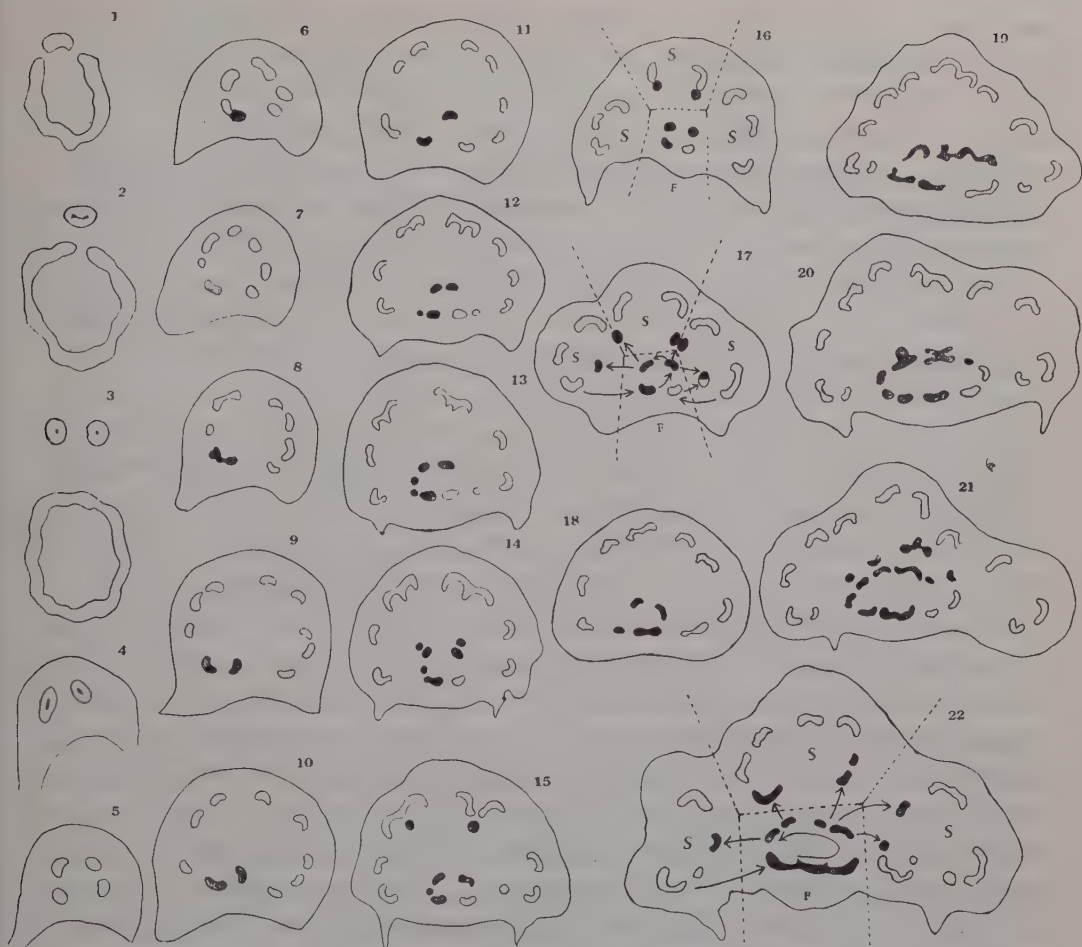
Figs. 1-16.   Serial cross sections of the phyllomophore of *Helminthostachys zeylanica*.
Fig. 17.   Cross section of another specimen, corresponding with Fig. 16.
Figs. 18-22. Serial cross sections of the phyllomophore of larger plant.
              Blacks are cognate strands from one adaxial bundle.

of the rizome.   This strand, now fell into the pith, becomes the origin of the medul-
lary strands.   Other vascular strands are also divided respectively and increase in
number, but it is rather rare that they make networks with adjacent strands.   Both
ends of the ◯-like arrangement of strands approach close together to make a circle.
These processes are seen in the lower parts of the phyllomophore.

Soon thereafter the medullary strand is divided into two, still beging involved
within the pith (Fig. 12).   Now the left end of this divided medullary strand comes
close to the strand which has been still located in the adaxial, left end of the ◯-like
arrangement.   Thus the medullary and adaxial strands are arranged to make an
inner small circle and will cooperate to form the vascular supply for the sporophyll
(Fig. 13).   While making such a circle, each of the medullary strands is divided
also in a periclinal (tangential) plane, separating a strand toward the abaxial

direction, and, by the aid of some abaxial members in the ◯-like arrangement of strands, contributes the vacular supply for the trophophyll or sterile frond (Fig. 14—16). If, on the other hand, the medullary strand originates from the end of the adaxial group of right side half in ◯-like arrangement it has to make a clockwise involution. In no case were there observed both of the two ends giving off medullary strands simultaneuosly. Whether the medullary strand originates from the right or the left, namely whether it makes a clockwise or an anticlockwise course of involution, seems to be proper to each individual plant. There are two or three buds at the base of the phyllomophore and they are enveloped with stipular appendages.

As these buds, which are going to follow the preceding shoot, are vascularized already to a remarkable extent, we may readily be able to observe three to four shoots in total if we make sections across the apex of rhizome, in the base of the present-time phyllomophore. Among seven specimens observed, two showed clockwise and four showed anticlockwise involution, while one has altered the vascular running from clockwise to anticlockwise in the last, youngest shoot. The writer has not yet been able to find out what determines the direction of the vascular involution, although he thinks some ecological conditions might not be ignored.

In smaller specimens, the vascular supply for the sporophyll consits of both adaxial ends ot the ◯-like arrangement in the phyllomophore. One of them is larger than the other and separates off a strand which runs spirally into the pith. The latter then separates off the strands for the trophophyll. In larger, well-developed specimens, on the other hand, the sporophylls are supplied with the vascular bundles which consist of only one of the adaxial strands running inward and spirally. Such is not a provisional case, but it represents a definite propensity. An example is shown in the figures (Figs, 18—22). Such method of branching have not yet been reported in any species other than *Helminuthostachys zeylanica*, either in *Ophioglossales* or other groups of the ferns.

Disscussion and Conclusion

The writer has been considering, before the completion of his work, the branching method of vascular supply for the sporophyll :of this species extra-marginal, deducing from the descriptions by Farmar and Freeman, and has held it as a significant character to distinguish *Helminthostachys* from other genera of *Ophioglossales*.

Nozu[9] (1955) said recently that this branching follows not the extra-marginal, but the marginal method. It is now certain, throught the observations by the present writer, that the method of the vascullar branching toward the sporophyll should not be called the "true" extra-marginal, but it is also certain that the method does belong to the extra-marginal in a wider sense rather than to the marginal, for the strand, which originates from the adaxial end, makes a spiral in-

volution and gives off the strands not only to the sporophyll but also to the trophophyll as well. At any rate, such could by no means be called the marginal method.

Nozu described that the vascular bundles in the phyllomophore of his *Helminthostachys* were arranged in a "concentric 'non-continuous' (discontinuos?) ring", as he figured in his report (p. 88, Fig. 3, B. 1—2c.). The present writer, however, could not observe any of such concentric arrangement, and he thinks that a careful inspection will make clear the involute arrangement which, at a glance, might be mistaken as concentric.

As for the medullary strands, the description that "a medullary bundle is derived, at the base of the phyllomophore, from bundles on the ring" seems to be rather vague. The writer believes that the medullary strand is derived from an adaxial end of the strands arranged like–◯, but it never will come out "from the bundles on the ring". This point must be very important for understanding the character of the vascular behaviours in this fern.

According to Nozu, there seems to have been recognized two types of medullary strands: the first type is derived "from bundles on the ring" and the second represents the strands which are spearated from the first type strands, namely the medullary strands for the trophophyll. He considers the first type more proper and the second provisional. As the present writer believes, however, it is very doubtful if it would make any sense to distinguish those two types, typical and provisional, for they must be related intimately with each other and may represent, with the cooperation of the whole strands, the modified extra-marginal method in all the specimens. That the medullary strands for the trophophyll depart secondarily from the so-called "first strands" is an intrinsic inclination in *Helminthostachys* and could hardly be considered provisional as it has been expressed by Nozu[9] (1955).

Fig. 23. Diagrams of vascular behaviours in juvenile plants.
a: *Sceptridium*,   b: *Osmundopteris*   c: *Helminthostachys*   d: *Helminthostachys*, somewhat larger plant than c.
Black parts show xylems.

In younger stages the vascular behaviour in the phyllomophore, as shown in Fig. 23, is common in all of the species, *Sceptridium japonicum* Lyon, *Sceptridium ternatum* Lyon, *Osmundopteris viginiana* Small, *Osmundopteris stricta* Nishida, and *Helminthostachys zeylanica* Hooker: the vascular trace for the phyllomophore is divided into two, or into four in somewhat larger plants, as it ascends the phyllomophore; but soon afterwards the two (or four) are united again to form a single bundle which at last will be divided into three

to run into the triphyllous (ternate) sterile frouds. Thus they have the common type of vascular branching while they are young, irrespective of their species.

As the plant grows larger any of the three different methods, i. e. the marginal, the extra-marginal, and the modified extra-marginal like in *Helminthostachys*, will be derived from the common, juvenile type. The uique branching in the phyllomophore of *Helmisnthostachys zeylanica* appears to be derived first from the marginal method to get afterwareds, the extra-marginal character. Then the fertile bundle becomes to be supplied more strongly with strands from one end of the ◯-like arrangement. Finally the sporophyll is supplied only with the adaxial strand from one of two ends of ◯. In other wards, a single adaxial strand makes an involute running into the pith and makes a circle of bundles by itself in the sporophyll.

This branching method in *Helminthostachys* is quite special and unique, and has never been found in other species of *Ophioglossales*. The writer, therefore, should like to call it the "**hetero-marginal method**", as it is distinguished with the tendency that the sporophyll is supplied mainly with a single, adaxial strand. The so-called marginal method, on the other hand, would more exactly be called the "**iso-marginal method**" as the writer proposes.

Thus the three kinds of branching method, iso-marginal, hetero-marginal and extra-marginal, could be established in the vascular supply for the sporophyll in the order *Ophioglossales*.

The vascular arrangement in the phyllomophore of *Helminthostachys*, having the medullary strands, resembles that of the rhizome and stipe of *Angiopteris* and *Danaea* which belong to *Marattiaceae*. Especially *Danaea* has a creeping rhizome and is much more like *Helminthostachys* in the mode of venation. These genera, however, have the true concentric arrangement of vascular bundles, and are quite different from *Helminthostachys* which has the involute arrangement. The medullary strands in *Danaea* originates from the adaxial strands and run in "zigzag spinal" (Brebner') to make up the true concentric ring, but the fronds in this plant, no doubt, are not made solid by the combination of fertile and sterile fronds, namely, this plant has no phyllomophore at all, and its resemblance to *Helmtnthostachys* therefore, must be only superficial.

Summary

The vascular branching for the sporophyll in the phyllomophore of *Helmintho-stachys zeylanica* was described. Certain supplements were given to the recent obser-vation by Nozu (1955) on the same species and his opinions were criticized.

The present writer proposed the term "**hetero-marginal method**", in contrast with marginal and extra-marginal method, to designate the vascular branching toward the sporophyll of *Helminthostachys zeylanica* as the vascular supply for the

sporophyll in this species has the tendency to be originating from one, instead of two, adaxial end of ◠-like arrangement of vascular bundles, and to run spirally into the pith of the phyllomophore.

The writer also proposed "**iso-marginal method**" to replace the so-called marginal method, and attempted to establish three types of the methods, in the phyllomophore of the order Ophioglossales, iso-, hetero-, and extra-marginal, in order to express more precisely the character of the vacscular branching.

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The Place of the Genus
Phyllorachis in the Sytem of Gramineae*

by Tuguo TATEOKA**

館岡亜緒： *Phyllorachis* 属（イネ科）の分類学的位置

Phyllorachis represents a monotypic genus first described by Trimen in 1879. It's single species, *P. sagittata*, inhabits Angola, Portuguese East Africa and Tanganika Territory in Africa. Although it is considerd to be one of themo st interesting genera from the point of view of phylogeny, it has not been studied either karyologically or anatomically. A sample of seeds collected last year in Portugese East Africa, Garuso, was kindly supplied by Dr. H. G. Schweickerdt, and germinated in our experimental garden. The present author was able to subject the plants to a karyological and morphological study. The results of his observations and some considerations on the relationships of this genus to the others are reported in this paper.

Observations

1. First leaf of the seedling

Avdulov (1931) pointed out that the first leaf of grass seedlings has a systema-

* Contributions from the National Institute of Genetics, No. 128.
** National Institute of Genetics, Misima, Sizuoka Pref., Japan.

tic significance; he distinguished two maintypes: Type I, first leaf elliptic or
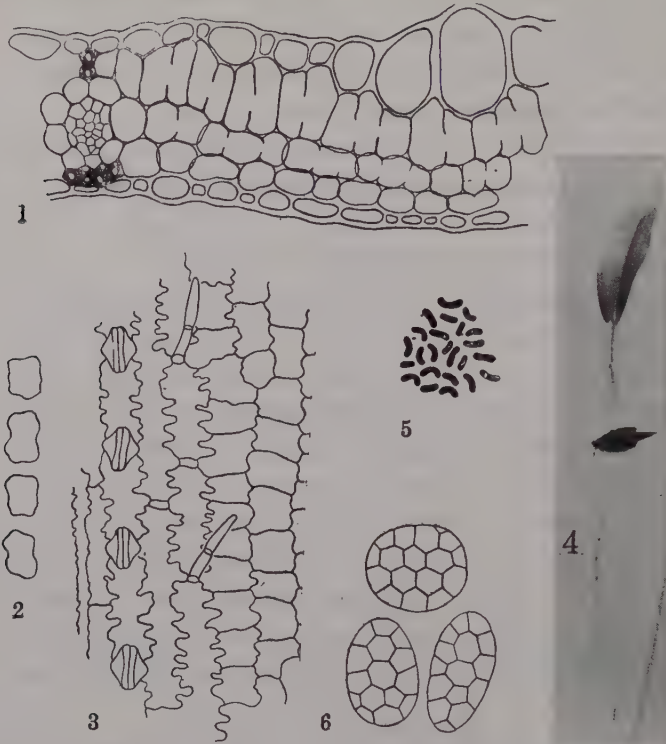
Phyllorachis sagittata Trimen　　Fig. 1.　A transverse leaf section ×
ca. 500.　Fig. 2.　Siliceous epidermis cells × ca. 600.　Fig. 3.　A part
of epidermis × ca. 300.　　Fig. 4.　Seedling × 1.　　Fig. 5.　Somatic
chromosomes × 2000.　Fig. 6.　Starch grains in endosperms × 150.

lanceolate, and horizontal or ascendent; Type II, first leaf linear and perpendicular.
The former type characterizes the members of Paniceae, Andropogoneae, Chlorideae,
etc., and the latter is shared by the members of Festuceae, Hordeae, Aveneae, Phala-
riedae, etc.　*Phyllorachis sagittata* belongs to Type I (Fig. 4).

2.　Starch grains of the endosperm.

Hubbard (1939) mentioned that the starch grains in the endosperm of
Phyllorachis sagittata were compound.　This is in agreement with the present
author's observation (Fig.6) who previously (1954, 1955) reviewed the systematic
significance of this character and found compound grains in a few members of
Paniceae, although almost all representatives of this tribe showed simple starch
grains.　He concluded that further developmental investigations on starch formatron
were required.　The compound grains of *Phyllorachis sagittata*, however, are typical,
suggesting that referring *Phyllorachis* to the tribe Paniceae is a mistake.

3.　Characteristics of epidermis and transverse sections of leaves.

The anatomical characteristics of grass leaves were studied and reviewed by

Avdulov (1931), Prat (1936), Potztal (1952), etc. They can be classified as follows.

Festucoid type—Chloroplasts are uniformly distributed throughout the mesophyll; epidermis lacks bicellular hairs and is characterized by siliceous cells that are round or rectangular. These characteristics are commonly found in members of typical festucoid genera.

Panicoid type—Chloroplasts are localized in a few cell layers surrounding directly the vascular bundles; epidermis includes dumb-bell-, cross-, or saddle-shaped siliceous cells and bicellular hairs. This type is divided into two following subtypes. Panicoid subtype, characterized by threadlike bicellular hairs and siliceous usually dumb-bell- or crossshaped cells, is found in members of Paniceae, Andropogoneae, Maydeae, etc. Chloridoid subtype shows thick clavate bicellular hairs and siliceous usually saddleshaped cells, and is found in the members of Chlorideae, Eragrosteae, etc.

Bambusoid type—Chloroplasts are uniformly distributed throughout the mesophyll, the cell layer surrounding directly the vascular bundles has a characteristic feature in that it lacks chloroplasts; epidermis has threadlike bi-(or several-) cellular hairs and siliceous cells similar to those of Panicoid type. Bambusoid type is represented by members of Bambuseae and considered to be the most primitive with respect to the anatomical characteristics of the leaf.

In the transverse sections of leaves of *Phyllorachis sagittata*, a cell layer surrounding directly the vascular bundles is almost devoid of chloroplasts; chloroplasts were uniformly distributed throughout the mesophyll, which consisted of sponge-like and palisade-like parenchyma (Fig.1). The epidermis has threadlike bicellular hairs and somewhat dumb-bell-shaped siliceous cells (Figs.2—3), According to the classification described above, *Phyllorachis sagittata* belongs to the Bambusoid type.

4. Chromosome constitution.

Root-tip cells of *Phyllorachis sagittata* show twenty-four small chromosomes[*] (Fig.5). This number suggests the basic chrosome number as 12 or 6; the meiotic behaviour was not observed. This finding seems to be very interesting concerning the phylogeny of *Phyllorachis*, since the basic chromosome number of 12 or 6 small chromosomes is found only in primitive grass groups such as Oryzeae, Arundineae, Bambuseae, etc.

Considerations

Bentham (1881) referred *Phyllorachis* to the tribe Paniceae. This view was followed by Hackel (1887), Chase (1911), Bews (1929), etc. However, Hackel (1.c.) himself remarked that the reference of this genus to Paniceae was doubtful. Hubbard (1939) expressed an opinion that this genus should constitute, together with

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[*] Root tips were fixed with Nawashin's solution, embedded in paraffin and cut at 15 micra. Crystal violet was used for staining. The figure was drawn wich the aid of an Abbe drawing apparatus.

Humbertochloa, having many similarities in common in gross morphology, an inde-
pendent tribe, Phyllorachieae. His opinion was based on a study of external mor-
phology and a few observations of anatomical characteristics of leaves and starch
grains. Hubbard's opinion was shared by Pilger (1954) who placed Phyllorachieae
under his Subfam. Festucoideae.

From the results of observations described above, the uncorrectness of placing
Phyllorachis in Paniceae or Chlorideae may be clearly pointed out as follows; 1)
almost all members of Paniceae or Chlorideae show polyploid chromcsome numbers
of basic 9 or 10, 2) members of Paniceae or Chlorideae have Panicoid type in the
anatomical characteristics of leaf. These features which characterize almost all
representatives of Paniceae or Chlorideae are not in agreement with those of
Phyllorachis.

On the other hand, *Phyllorachis* differs as follows from the typical members of
Festuciformes group in the characteristics described above: 1) members of Festuci-
formes group have large somatic chromosomes mostly showing polyploid numbers of
basic 7, 2) the type of anatomical characteristics of leaf in Festuciformes group is
usually festucoid, 3) the first leaf of the seedlings of Festuciformes group are
linear and perpendicular. *Phyllorachis* never shows these characteristics.

Phyllorachis sagittata must be a relic, considering the characteristics described
above as well as the features of gross morphology ananlyzed by Hubbard (1939).
Therefore, *Phyllorachis* together with *Humbertochloa* definitely should be treated as
an independent tribe. Hubbard (1.c) suggests the near relationship of Phyllorachieae
with Oryzeae, based upon some characteristics of gross morphology and the nature
of starch grains. Hubbard's suggestion is also supported by the karyological and
anatomical characteristics reported in the present study. A large majority of Oryzeae
show polyploid chromosome numbers of 12 as well as *Phyllorachis* and also resemble
Phyllorachis in the anatomical leaf characteristics. Pilgar (1954) placed Phyllora-
chieae in the Subfam. Festucoideae, and treated Oryzeae as an independent subfa-
mily, Oryzoideae. It seems desirable that more thorough studies are carried out
before deciding whether *Phyllorachis* belongs to Oryzoideae or must be treated as
a member of Festucoideae.

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組織切片によるヤヌスグリーンB還元と
ミトコンドリヤ染色の機構について

佐 藤 七 郎*

Sitiro Saro : On the Reduction of Janus Green B by Plant Embryo
Slices and the Mechanism of the Specific Stainiug of Mitochondria.

著者はツルナシインゲンの幼胚の切片をヤヌスグリーンB (JG-B) で生体染色してミトコンドリヤの分布をしるひとつのよりどころとした[3]。そのさい無気条件で色素の還元変色がおこることをみた。

しかしなにをさしてミトコンドリヤとよぶかについては議論のあるところで，かるがるしく断定することはゆるされない。著者は主として細胞学で形態学的知見にもとずいてなされたつぎの定義にしたがつて論をすすめてきた。今後もこれにならうこととする。すなわち有機溶媒にとけやすくオスミウム酸などの酸化剤で固定されたものはヘマトキシリンや酸性フクシンでそまり直径 1μ 前後の球形または棒形をなす。生細胞中では JG-B にそまり無気条件下ではこれを還元脱色する。以上の性質をそなえ，核，空胞，色素体内にはあらわれない大型細胞質顆粒である。これらの性質のうち JG-B でそまるだけでなくこれを還元することは油滴などと区別する重要な属性である。この原因はミトコンドリヤ中の高い脱水素酵素活性に帰せられる。

上記における還元変色が脱水素酵素によるものであることをたしかめるために定性的な実験をおこない，いくつかの結果をえたのでここに報告し，あわせて，えられた結果にかかわりのあるミトコンドリヤの特異的染色の機構の説について意見をのべる。

A. 還元変色の定性的研究

（1） 空気の条件——発芽前のツルナシインゲ

ン幼胚の切片をスライドグラスの上で JG-B (1：10,000) 水溶液にひたすと，ズイと原皮層がそまる。すぐにカバーグラスをかけると，ミズイロにそまつた切片はムラサキをへてボタンイロに変る。カバーの縁をワラップなどで封ずるとなおよい。長時間ののちには無色になる。カバーグラスをかけないでおくと，こいアイイロにそまるだけで色は変らない。しかしこうしてこくそまつた切片にカバーグラスをかけると，まもなく色が変つてくる。変色はいつもカバーグラスの中心部，つまり縁からとおい所からはじまり，カバーグラスの下に気泡がふくまれているときは気泡の囲りは変色がおくれる。

いつたんムラサキイロになつたときにカバーグラスをはがして空気にさらしてやると，切片はふたたびもとのアオイロにかえる。ボタンイロになつてからカバーグラスをはがしたのでは，もはやアオイロにもどらず，そのままの色でとどまる。さらに変色がすすんで完全に脱色して無色になつた切片では，空気にふれるとやはりふたたび色がついてくるが，ボタン色でとまり，それ以上にはもどらない。

JG-B のかかる変色，脱色および再発色は，JG-B の *in vitro* での還元，再酸化のさいにおこるそれとおなじ性質をもつているから，切片による変色，脱色および空気による再発色は，組織による還元および空気中の酸素による再酸化にちがいない。切片をあらかじめ熱でころしておくと還元はおこらないから，これには酵素がはたらいている。

カバーグラスをかけない切片や縁の近くにある切片，気泡の近くにある細胞で変色がおこらず，あ

* 東京大学理学部植物学教室細胞学研究室

るいは変色がおくれるのは、酸素による還元 JG-
B の自働再酸化および終末酸化酵素との競争があ
るからだろう。JG-B の酸化還元電位は低いから
微量の酸素でも還元がおくれる。

（2）　阻害および促進実験——このときは色素
は M/10 のリン酸緩衝液（pH6.8）にとかし（1：
10,000）これを対照混液とし、これに薬物をとか
しこんだものを薬物混液として、同一の胚からと
りだした切片に滴下して比較した。前処理の作用
をこころみるときは、とくにことわらないかぎり、
薬物はおなじ緩衝液にとかして前処理液とした。
このばあいには対照として、同じ胚からとつた切
片を緩衝液だけで前処理する。いずれのばあいも
基質はとくに加えない。比較は同じ幼胚からきり
とつて同時にしたてた切片どうしでのみおこな
い、ことなつた幼胚からの切片のあいだの比較は
さけた。混液を切片の上に滴下してすぐカバーグ
ラスをかけ、縁をワラツプで封じた。これは無気
条件とはいいきれないが、カバーグラスをかけな
いものと比べるときには無気ということにした。
室温（20°C ぐらい）に放置してかんさつした。
そのほかの条件は前の報告とおなじ。

（3）　熱——切片をあらかじめ 80°C の熱水に
10 分間つけると変色作用は不活性化され色素混液
をあたえてもただ青くそまるだけで変色できな
い。52°C、30 分でも不活性化され、切片ぜんたい
がウスアオイロにそまり、前形成層と他との区別
はあらわれない。

（4）　pH——pH 値 5.3 のばあいと 6.8 のば
あいとを比べる。90 分後に、pH 5.3 の方はまだ
ミズイロだが pH 6.8 の方はムラサキイロになつ
ている。すなわち酸性側では変色はほとんどすす
んでいない。

（5）　サク酸塩——pH 5.6 でサク酸緩衝液（W-
alpole）をつかつたばあいとリン酸緩衝液（Sören-
sen）をつかつたばあいを比べると、60 分ごに、
サク酸緩衝液をつかつた方はミズイロないしアオ
イロにとどまつているが、リン酸緩衝液の方はウ
スムラサキイロになつている。サク酸塩は阻害作
用をもつている。これ以外の実験にはサク酸緩衝
液はつかわない。

（6）　コハク酸——最終濃度 M/100 のコハク酸
ソーダをふくむ混液にただ切片をひたしただけで
は、えいきようが明かでない。しかし切片をあら

かじめ濃度の高い（0.6M）コハク酸ソーダ水溶液
にしずめ、水流ポンプで排気して細胞間隙にじゆ
うぶんに（85 分間）滲透させてから色素液をあた
えると、対照として同時間だけ蒸溜水を滲透させ
たものよりもはやく、20 分ごに差があらわれてく
る。コハク酸ソーダによつて変色が促進される。
しかし差はすくなく長時間ののちには同じ色にな
つてしまう。

（7）　マロン酸——M/100 の マロン酸 によつ
て変色はいちじるしく阻害される。70 分ごには対
照の方はアオムラサキからムラサキになつている
が、マロン酸を添加した方はにごつたアオイロで
差は大きく明かである。マロン酸とコハク酸塩を
同時に加えておく（いずれも M/100）と、マロン
酸のこの作用はあらわれなくなる。あらかじめ M
/100 のマロン酸をふくんだ緩衝液で 55 分間前処
理してもこれをマロン酸をふくまぬ混液にうつす
と、マロン酸の阻害作用はほとんどみとめられな
くなる。このようにマロン酸はつよい阻害作用を
もつている。

（8）　青酸——JG-B の変色、脱色は無気条件
下でおこり、また青酸はそれを促進するといわれ
ている。[7,8] しかしこの実験では、KCN の M/1000
をふくむ薬物混液をもちいると、カバーグラスを
かけておけば変色はおこるが、しかし KCN をふ
くまぬ対照混液にくらべて明かにおくれる。つま
り無気条件では青酸は変色を阻害する。カバーグ
ラスをかけないでおくと、M/100 という高い青
酸の濃度でも変色はまつたくおこらない。これは
青酸をふくまぬばあいとおなじである。切片は時
間がたつにつれてますますこくそまつてくる。し
かしこうなつたものもカバーグラスをかけると、
しばらくして変色がはじまる。はじめから青酸を
ふくまぬ対照混液にひたし、しかもカバーグラス
をかけずに青くそまつた切片も、カバーグラスを
かけさえしなければ、たとえ M/100 の青酸をあ
たえても変色はおこらない。すなわち青酸の有無
にかかわりなく空気にふれていれば変色はとめら
れる。

（9）　その他——モノヨードサク酸（M/1000）
をふくむ混液では阻害はみられない。しかしこれ
を pH 7.0 の M/10 リン酸緩衝液に M/1000 にと
かした液に切片を 60 分しずめて前処理したあと
ではごくよわい阻害作用があらわれた。さら

に，とうして前処理（40分）した切片を薬物混液（M/100）でそめると，変色はつよく阻害され，約80分ごとに対照はウスムラサキになつているのにアオイロのままであつた。

アヒ酸（約M/300）も阻害作用をもつている。弗化ソーダ（M/100）はえいきようがみとめられない。

硝酸ナマリ（M/10）で60分前処理すると変色能力はまつたくなくなる。

フエニールウレタン飽和水溶液，エチルウレタン（M/100）も作用なし。

（10）　考察──この実験でのJG-B還元に直接に関与している酵素がどのような酵素であるかは不明である。しかしながらコハク酸添加による促進，マロン酸その他による阻害の事実から，この還元になんらかの形でコハク酸脱水素酵素が関与することは推定される。酸化還元電位からみればコハク酸脱水素酵素が直接にJG-Bの還元に当るとはかんがえがたく，そのあいだにはいくつかの水素伝達体が介在するものとおもわれる。前形成層がそまらないのはミトコンドリヤが分布していない[3]ためであろう。このぶぶんにはコハク酸脱水素酵素活性が高い[1,2]から，色素が急速に還元脱色されるために着色しない可能性も考えられるが，JG-Bの酸化還元電位が低いうえにNadi反応もつよいにかかわらず，有気条件下でもそまらないから，そのような可能性はすくないとおもわれる。

有気条件下で変色がおこらないのは色素の自動酸化のためであろう。青酸の作用については従来の報告と一致しないし，青酸がJG-Bと沈澱をつくる事実もあるので，その原因については実験をかさねなければ結論はだせない。ただテトラゾリウム塩還元でコハク酸脱水素酵素活性をしらべたときにも阻害があつたことを附記する。

B. ミトコンドリヤのJG-B染色の理論について──Cooperstein, Lazarov and Patterson (1935[5]) はJG-Bの吸着にかんしてミトコンドリヤの特異性はなく非顆粒性の細胞基質も同様にこれを吸着するという結論を，細胞質の各構造要素を遠心分割したものについての実験からみちびいている。そしてこの結論とJG-B染色が無気条件あるいは青酸の存在下で阻れるという観察にもとずいて，ミトコンドリヤのJG-B染色の機構にかん

して一説をたてた[6]。それによると生細胞はミトコンドリヤその他の細胞質顆粒と非顆粒性細胞基質（ヒヤロプラズマ）とをとわず，JG-B液中で一様に色素を吸着する。しかし脱水素酵素によつてつねにこれが還元される傾向にある。ところがミトコンドリヤはチトクロームオキシダーゼを多量にふくんでいて酸化電位が高くJG-Bを還元しない。その結果としてミトコンドリヤだけが青くそまつてのこり，他が脱色しさるのだという。

無気条件下や青酸の存在下でミトコンドリヤがそまらないのは，チトクロームオキシダーゼの作用が停止して，JG-Bが還元脱色されるためと説明される。

しかしこの説にはつぎのような難点がある。

第1。ミズカビの菌糸，トチカガミの根毛，高等植物の表皮などでの観察では，JG-B溶液中ではじめに着色するのは明かにミトコンドリヤのごとき顆粒であつて，非顆粒性の細胞基質をもふくめた全細胞質が均一にそまるという事実はない。

第2。もし上の説のように，いつたん細胞基質がそまつてから還元脱色するとすれば，その過程でムラサキやボタンイロの段階をへるはずであるが，このような事実はない。

第3。Lazarov and Cooperstein[4] は均一化し遠心分離して単離したミトコンドリヤと細胞基質のJG-B吸着能を比較して差をみとめられずとしているが，分離操作の過程でタンパクの色素吸着性が変らないという保証はない。Goldacre[9] は振盪によつてタンパクの色素（中性赤）吸着性が変ることを実証している。したがつて上記の実験だけでミトコンドリヤのJG-B染色の特異性を否定するのは根拠薄弱といわなければならない。

第4。かれらの説にしたがえば本実験ではミトコンドリヤをふくまない前形成層も他と同様にそまらなければならないが，事実はそれとことなる。

結論──JG-Bによるミトコンドリヤ染色は，やはり，従来のように特異的な吸着をもつて説明さるべきであろう。

おわりに本実験のために各角度から有益な意見をよせていただいた研究室の抄読会のかたがたならびに小倉安之博士に感謝します。なお本報告は1955年10月に日本植物学会第20回大会において発表したものである。

Summary

Janus green B stains the periderm and the pith of hypocotyl and radicle of un-germinated seeds of *Phaseolus vulgaris*. The procambium, which contains no mito-chondria, is not stained from beginning to end of the procedure unless the cells are living Under a coverglass, the stain reduced and decolorized.   This reduction is inhibited by heating (52°c for 30 min.), low pH, acetate buffer (M/10), malonate monoiodoacetate (M/1000), arsenite (M/300), and $Pb(NO_3)_2$ (M/10), and accelerated (M/100), by succinate (6M/10).   These inhibitions and acceleration show that succinic dehydrogenase plays a role in the process of the reduction of Janus green B, presumably in directly under the present experimental conditions.

Cyanide (M/1000) also inhibits the reduction.

Without coverglass, i. e. in complete aerobic condition, the reduction dose not occur whether cyanide is present or not.

The hypothesis of L Aazarow and Cooperstein on the mechanism of the specific staninig of mitochondria was criticized on the ground of the above stated experim-ental data and some theoretical considerations.

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花 粉 の 生 理 学 的 研 究 IX
花 粉 粒 内 の 澱 粉 と 糖 に つ い て[*]

岩 波 洋 造[**]

Yōzō IWANAMI : Physiological Researches of Pollen IX
The Starch and the Sugar in the Pollen Grain

さきに *Impatiens* の花粉について，培養基上で澱粉粒の消長がみられることを報じたが，[1] 花粉粒内の炭水化物は直接花粉の生長に関係すると思われるので，今回は花粉の形成過程における澱粉粒の消長，及び花粉粒内の種々の糖類について調べた結果を報告する。

1. 花粉粒の形成過程における澱粉粒の消長。

花粉が花粉母細胞から個々の花粉になるまでの諸過程において，しばしば細胞中に澱粉粒の存在がみられることは，すでに桐野氏[2]らによつて観察されている。しかしながらこれら澱粉粒の消長の意味，及び澱粉粒の消失後の形成物質などについては，まだ明らかにされていない。筆者はまず花粉粒分裂，及び個々の細胞の大きさの変化と，澱粉粒の消長との時期的な関係を詳細に調査した。

実 験 1

Tradescantia reflexa Rafin. の花において，同一花の葯中の花粉は花粉粒分裂，澱粉粒の出現
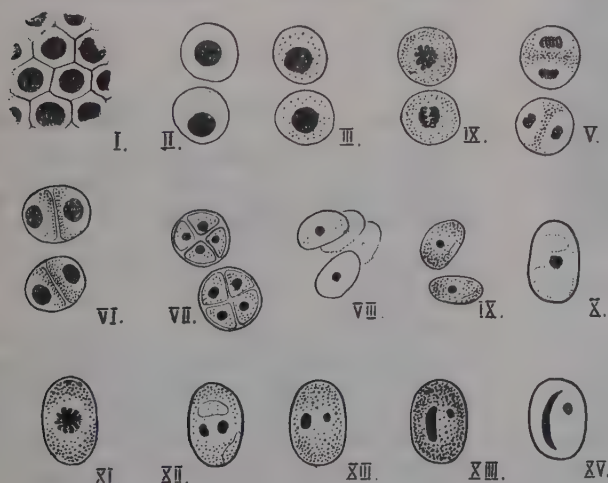
fig. 1 花粉粒の形成時における澱粉粒の消長
（*Tradescantia reflexa Rafin.*）

の割合がほゞ同じであることを最初に確かめたので，1枚のスライドグラスに同じ花から2ケ所に花粉をとり，一方は酢酸カーミンによつて核の分裂過程を調べ，他の一つは抱水クロラールョード液によつて澱粉粒の有無を調べ，同時にミクロメーターで花粉（細胞）の大きさを測定した。

花粉母細胞から分れて個々の花粉に成熟するまでを15の過程に分け，これらの過程をI〜XV で示した。したがつて XV は開葯時の花粉を意味している。fig. 1 はこれらの過程における澱粉粒の消長を示している。即ち澱粉粒は多くの過程でみられるが，I〜II, VII, X, XV の各過程においてはこれを欠いていることがわかる。

fig. 2 はこの過程を通じての細胞の大きさの変化と澱粉粒の消長との関係を示している。このグラフからみられるように，細胞が急激にその大きさを増す時 (I〜II, X) は何れも澱粉粒が消失し一定の大きさになると次第に澱粉粒を増加していることがわかる。又 VII の過程で澱粉粒が消失しているのは，figs. 1 でみられるとおり，丁度4分子花粉の形成される時期であつて，fig. 2 においてこの少し前(VI〜VII)にグラフの線が2つに分れているのは，上の線は4つの花粉に分れる前のものゝ長径を示し，下の線は新細胞の長径を示しているからである。したがつてこれら3者の場合は何れも急激に炭水化物を要求する時期であるし，澱粉粒以外の部分のョードの反応が殆どみられないことからも，この時期の盛な代謝
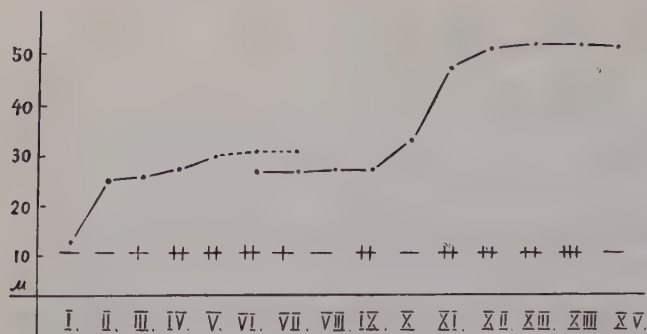
fig 2　花粉粒の形成時における細胞の大きさ
　　　（長径）の変化と澱粉粒の消長との関係
……澱粉粒なし　＋, ╫, ╫╫…澱粉粒あり

の材料として澱粉粒が使われたとみてよいであろ
う。たゞ残された 1 つの過程，即ち最後の XIV〜
XV の過程については，すでに花粉粒の大きさも
決り，形態もとゝのつているにもかゝわらず，開花
の時期に至つて急激に澱粉粒を失つているのでこ
の時の消失は他の 3 者とは意味を異にしていると
思われる。しかもその直前の過程，即ち XIV の
ものの中には，他のどの過程のものよりも多量に
澱粉粒を有している。

これと同様な現象，即ち蕾の時期において多量に
有している澱粉粒を開花の時期において急激に消
失することは，*Tradescantia* 以外にも多くの花
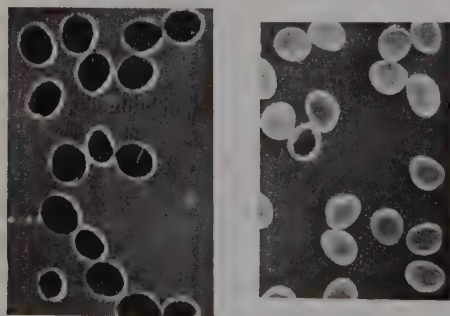
fig. 3　*Lilium longiflorum* Thumb. の花粉粒
　　　内の澱粉の有無
　　　左…蕾の花粉　右…開葯時の花粉

粉でみられる。fig. 3 の写真，及び fig. 4 は *Li-
lium longiflorum* Thumb. 及び *Lilium auratum*
Lindl., *Antirrhinum majus* L., *Gladiolus gran-
davensis* Van Houtt. の各花粉の蕾と開花時の澱
粉粒の有無を示している。

このように同一細胞中で澱粉粒が
急激に消失していることは，花粉が
発芽時の炭水化物の要求にそなえ
て，これを糖にかえているのではな
いかと考えることができる。このこ
とはヨードの反応が，澱粉粒の消失
につれて花粉粒全体が黄褐色になる
ことからも推察されるが，次に両者
の花粉の滲透圧の高さを調べてこれ
を比較した。
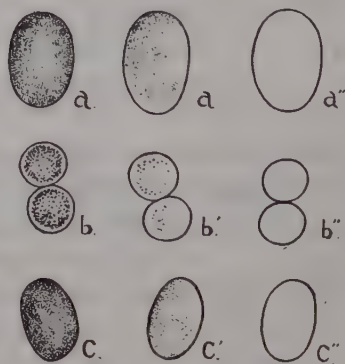
figs. 4　開葯時近くの澱粉粒の消失
　　a-a” …*Lilium auratum* Linld.
　　b-b” …*Antirrhioum majus* L.
　　c-c” …*Gladiolus grandavensis* Van Houtt
　　(a”, b”, c”,は何れも開葯時の花粉)

実　験　2

Sucrose のいろいろな濃度の液（1mol〜0.55m
ol.）の中に *Tradescantia reflexa* Rafin. の XIV
と XV の花粉を入れ，10 分後にこれを鏡検した。
原形質分離を起しているものを＋で示し，起して
いないものを−で示して両者の比較をしたものが
表 1 である。

表 1 の結果から澱粉粒を消失した花粉は，これ
を失う前の花粉に比べて可成り高い滲透圧を有し
ていることがわかる。したがつて前記ヨード反応
の結果と考え合せると，ほゞ花粉が開花時に澱粉
粒を糖の形にかえて貯えていると考えられるの
で，次に花粉粒内の糖について調査を行つた。

II　花粉粒内の糖の種類とその変化。

Table 1.　Sucrose の液中における *Tradescantia* の花粉の原形質分離.

mol	1	0.95	0.9	0.85	0.8	0.75	0.7	0,65	0.6	0.55
XIV	++ ++	+++ +++	++ ++	+++ +++	+++ +++	+++ ++-	+++ +--	--- ---	--- ---	--- --
XV	++ ++	+++ +++	+++ ++-	++- +--	--- ---	--- ---	--- --	-- --	-- --	-- --

+……原形質分離をしたもの　-……原形質分離をしないもの　各附号は 5個の花粉を示している。
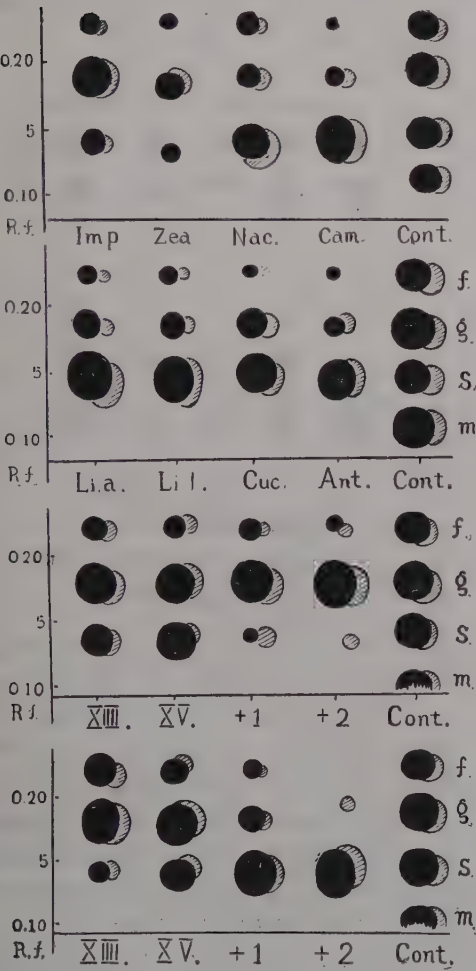
fig. 5.　種々の花粉粒に含まれている糖類
　第1段，第2段─開花時の花粉粒中の糖
　Imp. — *Impatiens Balsamina* L., Zea—
　　Zea mays L.
　Nac. — *Narcissus Jonquilla* L., Cam—
　　Camellia japonica L. Lia—*Lilium aura-*
　　tum Lindl.

Lil — *Lilium longiflorum* Thumb., Cuc
　— *Cucurbita moschata* Duch., Ant —
　Antirrhinum majus L.
第3段 — *Impatiens Balsamina* L. の花粉
　粒の成熟と糖の変化。
第4段 — *Lilium longiflorum* Thumb. の
　花粉粒の成熟と糖の変化。

　花粉が糖を有していることは，すでに Ander-son,[3] 市川氏,[4] 早瀬氏[5] らによつて 2,3 の種について知られているが，糖の種類やその時期的な変化に関する研究はまだ行われていないようである。たゞ最近志佐氏ら[6] がペチュニヤの葯を花粉と共にすりつぶし，この中に glucose, sucrose, fr-uctose があることをみている。筆者は種々の花粉をペーパークロマトグラブにかけて，その中に含まれる糖の種類，及び花粉の生長にともなう糖の変化について調査を行つた。

実　験　3

　開葯時の花粉を約0.02g づつとつて，クロマトグラブで糖の種類を調べた。

　用いた花粉は (*Impatiens*)*Balsamina* L., *Zea Mays* L., *Narcissus Jonquilla* L., *Camellia jap-onica* L., *Lilium auratum* Lindl., *Lilium longi-florum* Thumb., *Cocurbita moschata* Duch., *Antirrhinum majus* L. などの花粉で，これらを80％アルコール 0.5cc の中ですりつぶした後，1cc の蒸溜水を加えながら遠心分離器で固型物をとり除いた。上澄液をガラスの蒸発皿にとつて 45°c の乾燥器中で乾燥させた後，無水ピリヂン 0.1cc に溶かし直してこれを0.06cc づつ濾紙に与えた。

　展開剤はブタノール酢酸液 (5:1:2) を用い，呈色剤はベンチヂン呈色剤を主として使い，レゾルシン呈色剤を併用しつゝ糖の種類を調べた。

　展開装置は 10×40cm の濾紙を 4板同時に平板のまゝ使用できるよう考案した上昇式で，濾紙は

東洋濾紙 No.50 を使つた。

　16 時間展開後に得られた結果が fig. 5 の上から 1 段目と 2 段目に示されている。結果は何れの花粉粒の中にも glucose, fructose, sucrose の 3 種類の糖が存在していることを示している。更に量的な差異に注目すると，これらの花粉の中で前 2 者は glucose を主として含み，他のものは sucrose を主として含有しているかに見られる。（クロマトグラムが二重に記されているのは二回の結果を記入したものである。）

実 験 4

　花粉粒中で澱粉粒の消長がみられると同様に，糖の種類も花粉の成熟度によつて違つていることが当然考えられるので，総ゆる点で対照的であると思われる *Impatiens Balsamina* L. と *Lilium longiflorum* Thumb. の 2 種類の花粉について，澱粉粒の消失の時期，及びその後の花粉粒内の糖の種類の変化を調査した。

　2 種の花粉の XIV 及び XV の花粉と，XV の花粉をシャーレの中で 24 時間，48 時間保存したもの（24 時間後のものを +1 で示し，48 時間後のものを +2 で示した）を前述同様の方法でペーパークロマトグラフにかけて調べた結果が fig. 5 の 3 段目 (*Impatiens*) と 4 段目に (*Lilium*) 示されている。

　即ち *Impatiens Balsamina* L. の花粉の糖は，時間の経過と共に次第に glucose に変り，一方 *Lilium longiflorum* Thumb. の花粉の糖は次第に sucrose に向つて進んでいることがわかる。このことは次の実験の結果と共に *Impatiens* の花粉が培養基にまかれてから直ちに発芽を始めるに対して，*Lilium* の花粉は 60～90 分もの間発芽を行わないことについて，或る種の示唆を与えてくれる。

実 験 5

　花粉が培養基にまかれてから一定の期間発芽を行わないこと，即ち一定の発芽時間を花粉が有することは，*Lilium* やその他の多くの花粉でみられるが，この発芽時間の間に花粉中の糖は変化すると考えられる。そこで花粉を培養して糖の種類を調査した。

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*　ベンチヂン 0.5g を 100cc の氷酢酸に溶解し，これに 40%（W/V）トリクロル酢酸水溶液 10cc とエタノール 80cc を加えたもの。

Impatiens の花粉は先述の如く発芽時間が極めて短いので，*Lilium longiflorum* Thumb. の花粉と，やゝ特殊性をもつ花粉ではあるが 30～40 分の発芽時間を有する *Camellia japonica* L. の花粉を lactose 5% の寒天培養基で培養し，時間の経過と共に培養基上から花粉だけをとつて前記同様にして糖の種類を調査した。ここで lactose を用いたのは培養基の滲透圧を調節する為であり，lactose が花粉の含む糖の中にないこと，花粉が lactose を容易に分解，吸収しないことを別の実験（未発表）で確かめているからである。
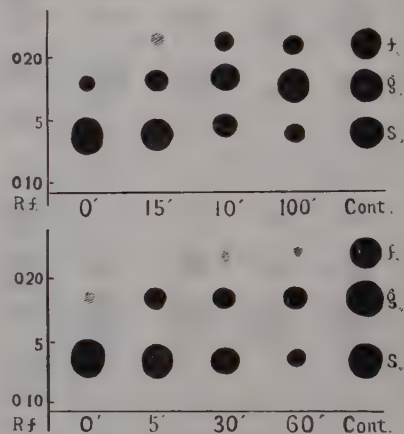
fig. 6　培養基上の花粉の糖の変化
　　　上段 — *Lilium longiflorum* Thumb.
　　　下段 — *Camellia japonica* L.

　結果は fig. 6 に示されている。（lactose は除く）即ち *Lilium* の花粉は時間と共に次第に sucrose を glucose と fructose に分解し，発芽前には glucose が多くを占めるようになり，先述の *Impatiens* の花粉とほゞ同様の傾向を示すようになる。また *Camellia* の花粉では sucrose が次第に減ずることは明かであるが，glucose の量はそれ程増加していない。ただ *Camellia* の花粉は 30～40 分ですでに花粉管を伸し始めているから，この事を考慮に入れねばならないし，この時花粉管の材料として直接使われた糖についてもこゝでは不明である。したがつて今回はこれら花粉の中の糖の変化をそのまゝこゝに報告しておく次第である。

Summary

1) The digestion and deposition of the starch grains in the growing pollen of *Tradescantia* were observedaand figured. (figs. 1)

2) In the immature pollen of *Tradescantia*, *Lilium*, *Antirrhinum*, *Gladiolus* and some other species the storage starch were found abundantly, but with the progress of maturation the starch grains became empty. (figs. 1, 3, 4)

3) The mature pollen of *Tradescantia* occured plasmolysis more easily than immature pollen (table 1). The result indicate that the storage starch are transformed into sugar.

4) The sugars in the pollen of *Impatiens*, *Zea*, *Narcisus*, *Camellia*, *Lilium*, *Cucurbita*, *and Antirrhinum* were investigated by mean of paper chromatography. Sucrose, glucose and fructose were found in the respective pollen grains (fig. 5).

5) On tne artificial culture media sucrose in the *Lilium* and *Camellia* pollen gradually removed, than glucose and frucotose increased (figs. 6).

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本 会 記 事

昭和30年度会計決算報告（昭和30年 4月から昭和30年12月まで）*

収入の部		支出の部	
会　　　費	484,620	出 版 費	617,564
予約購読料	58,420	発 送 費	69,058
一 部 売	20,470	編集関係費	27,340
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前年度繰越金	231,901	次年度繰越金	257,010
総　　　計	1,169,271	総　　　計	1,169,271

* 会則第13条の変更によつて31年度から会計年度は 1月に始まるので30年度は 4月から12月までの9ヶ月間の会計になります。

中部支部；第41回例会（於　瑞穂区名大教養部（旧八高）図書館）
微生物の窒素代謝について（綜説）……岩崎秀一（名大理）マリゴケについて　　　高木典雄（名大，教養）スライド投影，尾瀬の植物 ……高木典雄

北海道支部；第34回例会（於　北大理）トマト，イヌホウズキの接木世代に於る2，3の変化……増淵法之（北大理）　水稲における冷害研究の現状 ……島崎佳郎（北農試）

東北支部；日本植物学会東北支部報第 8 号（1955，12）を発行して支部大会の講演要旨，昭和29年度会計報告，昭和30年度会計中間報告，支部内規，寄稿，名簿などをのせていますが植物学雑誌と同版の印刷物です。講演名と寄稿の題名とを掲載致します。
ネモトシヤクナゲの複弁花の由来について ……小林四郎（福島一中）　山形県の野生桜について……結城嘉美（楯岡高）　福島県のサクラについて……小林勝（福島大学芸）　サクラ属の双胚に就て……松田孫治（鷹巣農高）　東北地方産カラマツサウとツルボについて……菊池政雄（岩手大学芸）　*Cupressus sempervirens* L. の胚発生 ……杉原美徳（東北大教育）*Salix triandra* L. と *S. subfragilis*. Anderss. の葉の比較解剖……千田貞蔵（仙台二女高）花粉内核分裂について二，三の問題 ……中沢潤（弘前大文理）　清酒酵母の胞子形成能について……倉石衍，山本総，三戸信人（東北大理）　三菱油戸炭田の花粉分析 ……高橋信雄（新庄北高）　庄内地方の森林群落……吉岡邦二（福島大学芸）　生活形より見た山地草原の特性……飯泉茂，（東北大理）菅原亀悦（岩ケ崎高）　羊歯類前葉体における偏光効果……伊倉伊三美（山形大教育）　大豆めばえの呼吸……安部守（山形大文理）　邦産かき属の異型果の発生学的研究（予報2）……大伍進（福島高）アロミセス菌二種の生育条件について……加藤君雄（秋田大学芸）　イネ鞘葉の脱水素酵素 ……金沢保（東北大.理）　森林帯の間帯並びに維移帯について……太田哲（宮城農短大）　福島県のサクラに就いて ……小林勝（福島大学芸）山形県の野生桜について……結城嘉美（山形楯岡高）福島県大滝根山地帯の蘚類……樋口利雄（福島信夫高）

九州支部；第37回例会（於九大理）　中国におけるアサクサノリ糸状体の研究紹介……吉田忠生（九大理）　福岡県の生葉上苔類（予報）……尼川大録（修献館高）　阿蘇久住両山塊の植物概況……鈴木時夫（大分大学芸）

Studies on the Adaptation of Yeast to Copper XIII.
Effect of Copper on the Amino Acid Pool[*]

by Tetsuo MURAYAMA, Mutsuo IMAI and Joji ASHIDA[**]

村山徹郎・今井六雄・芦田譲治：　酵母の銅に対する適応的変異現象の研究 XIII.
アミノ酸プールに及ぼす銅の影響

In what respects does the copper-trained substrain differ from the parent strain ? How can the former grow easily in the presence of copper ?　These problems should be approached from various respects.

Minagawa[1] found that the inhibition by copper of fermentation is less with the resistant cells than with the parent cells.　Naiki *et al.*[2] found that the former has a much higher copper-binding power than the latter, and Arakatsu *et al.*[3] have suggested that glutamic and aspartic acids favour the growth of the parent cells in the presence of copper.

Growth must be supported by as much protein synthesis as is required, whether growth occurs in a beneficial medium or in an injurious one.　When cells grow in the presence of copper, which may attack proteins and intermediates of nitrogen metabolism, aberrations in the nitrogen metabolism are highly conceivable.　The present authors have begun with a study in the amino acid pool.

The present paper reports the disturbances found in the amino acid pool of cells grown in some copper media.　The paper begins with descriptions of the preliminary tests made to find proper procedures for the study of amino acid pool using paper chromatography.

Material and Methods

The strain of *Saccharomyces ellipsoideus* used in this experiment was the same one as used in the experiments reported previously[4,5,6].　The copper resistant substrains which are obtained by successive subcultures on the media that are poisoned with 1 and 4 mM/1 cupric sulfate are called R_{1b} and R_{4b}, respectively.　These strains are brown coloured on the copper media.　When these strains were transferred to the normal medium to which no copper was supplemented, the growth was not coloured brown.　But they were found still resistant to copper when returned to the copper medium.　R_{1b} and R_{4b} cultured on the normal medium are called $R_{1b(0)}$ and $R_{4b(0)}$, respectively.

The culture medium used was what was called MH* in the preceding papers. Copper medium was prepared by adding a calculated amount of sterilized cupric sulfate solution to the sterilized MH medium at room temperature, the pH before inoculation being adjusted to 5.2 with NaOH. The incubation temperature was 30°C.

Total nitrogen was determined with a modified micro-Kjeldahl method described by Y. Yagi[7, 8]. Following heating with 50% sulfuric acid, all nitrogenous compounds were oxidized to ammonium sulfate, with hydrogen peroxide as a catalyzer. After cooling, excess sulfuric acid was neutralized with NaOH and sodium borate. Ammonium was converted to nitrogen gas by an excess of NaOBr. The remaining NaOBr was converted to NaBr by addition of KI. And the produced I_2 was titrated with sodium thiosulfate-starch solution as an indicator. Since 1 atom of organic nitrogen corresponds to 3/2 of NaBr, to 3/2 of I_2, and hence to 3 of $Na_2S_2O_3$, the nitrogen content in mg of the original sample is given by $\frac{14.01}{3}\cdot$ (Normality of $Na_2S_2O_3$ solution) $\cdot$ (V_0–V), where V_0 and V represent the volumes in ml of thiosulfate solution needed for the titrations of the blank and of the sample, respectively.

By this method, while the trouble of distillation is saved, an accuracy over 98% is expectable in the range of 5–50 microgram nitrogen per test aliquot.

For the identification of amino acids, the ascending two-dimensional paper chromatography was used. The first solvent was phenol containing 15% of water, and the second one was 30% water-saturated lutidine or water-saturated buthanol containing 1/5 by volume of glacial acetic. The development was made at room temperature in summer and at 25°C in winter. For the detection of amino acids, 0.15-0.2% ninhydrin acetone solution was sprayed over the chromatogram which had been kept at 90-100°C for 5 minutes. Relative amounts of amino acids were guessed roughly by the area and the colour density of each spot.

Sample solutions for paper chromatography were prepared as follows. Cells in the stationary phase of growth were harvested from liquid cultures. According to the results of the preliminary experiments reported below, the cells were washed four times with M/15 KH_2PO_4 solution, and then once with distilled water. After washing they were extracted with 70-80% (v/v) ethanol without being dried. An equal volume of chloroform was added to this extract[9], shaken, and supernatant was collected. This was condensed in a vacuum to ca. 4.0 mg nitrogen per ml. Of the total extractable nitrogen, 13% remained in the chloroform-ethanol phase. This fraction was, however, found to contain no amino acids when condensed further and tested by paper chromatography[10].

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* Cane sugar 100g, KH_2PO_4 5g, peptone (Funai) 5g, $MgSO_4 \cdot 7H_2O$ 1g, distilled water 1,000 ml, malt extract (Be. 80) 360 ml.

Results

1. Preliminary test for washing procedure. Amino acids which come directly from the culture medium should be washed out. But repetition of washing must necessarily be accompanied by a loss of constituents of the amino acid pool. Hence in order to find a suitable number of washings, the amount of organic nitrogen successively liberated from cells by repeated washing was determined by the modified micro-Kjeldahl method. The cells used were of the parent strain, harvested from a 48-hour culture in $1l$ of MH medium, the dry weight being ca. 5 g. They were washed with 50 ml of KH_2PO_4 each time. The nitrogen content of the first eluate was about 200 mg per g dry cells. Relative amounts of nitrogen in successive eluates are given in Table 1.

Table 1. Organic nitrogen liberated from cells by successive washing, in percentage of that in the first eluate.

No. of washing	1	2	3	4	5	6	7	8
Nitrogen content of eluate	100	39	30	17	15	12	11	11

The results showed that the liberation of nitrogenous matter from cells fell to a low and constant level after the fifth washing. Hence in the main experiment, cells were washed five times with KH_2PO_4 solution followed by a washing with distilled water. Distilled water was used lastly to wash out the salt which might interfere with chromatographic development.

2. Preliminary test for extraction procedure. In order to know the number of extractions necessary for the complete depletion of intracellular extractable amino acids, nigrogen contents were determined of the successive extracts of cells by 70-80% ethanol. The cells used were of the parent strain in the lag, in the log and in the stationary phases, as well as of R_{4b} and $R_{4b(0)}$ in the stationary phase. To an amount of cells which would be about 150 mg if dried, 100 ml of ethanol was added, shaken a while, and left standing at room temperature. Then the same volume of ethanol was renewed every 24 hour. The dry weight of the cells used was determined after the

Table 2. Amounts of nitrogen successively extracted, the percentage distribution in each series being given in brackets.

Strains	Phase of growth	No. of extraction (mg./100 ml. extract)				
		1	2	3	4	Total
Parent	Lag	22.0(91)	2.0(8)	0.3(1)	0	24.3(100)
"	Log	7.9(78)	1.5(16)	0.4(4)	0.2(2)	10.0(100)
"	Stationary	16.2(79)	3.1(15)	0.8(4)	0.4(2)	20.5(100)
$R_{4b(0)}$	Stationary	14.8(78)	3.0(16)	0.75(4)	0.38(2)	18.9(100)
R_{4b}	Stationary	8.0(53)	3.9(26)	2.1(14)	1.0(7)	15.0(100)

last extraction. The nitrogen contents of successive extracts per mg of this dry weight of cells are shown in Table 2. Except R_{4b} cells, which had been harvested directly from the copper medium, the extraction seems to be practically adequate by three times.

In the following experiments, therefore, three successive extracts, except R_{4b}, were gathered and used for chromatographic tests. In the case of this copper-grown culture, extraction was repeated four times, and the extract was bubbled with H_2S gas to trap copper.

In the log phase of the parent culture the total amount of extracted nitrogen was less than that in the other two phases. This may perhaps be due to relatively rapid consumption of free amino acids compared with their production. In the stationary phase, the extracted nitrogen is less with cells grown in copper-medium than those grown in the normal medium.

3. Amino acid composition of cell extract. Representative chromatograms obtained are illustrated in the figure. Relative amounts of amino acids guessed by areas and colour densities of spots are shown in Table 3.
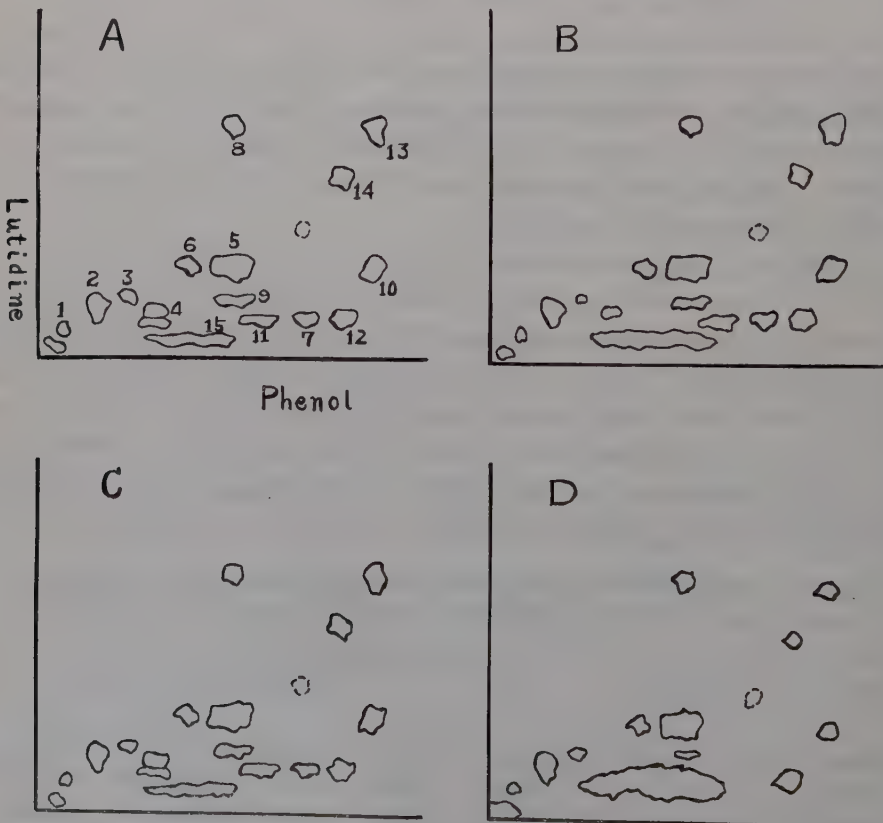
Figure. Paper chromatograms of alcohol extracts of cells at the stationary phase of growth. A: The parent strain; B: R_{1b} cultured in 1 mM-Cu-MH; C: $R_{1b(0)}$ and $R_{4b(0)}$ cultured in the normal MH; D: R_{4b} cultured in 4 mM-Cu-MH.

Table 3.　Amino acid composition of extracts from cells of the parent, R_{1b}, R_{4b}, $R_{1b(0)}$, and $R_{4b(0)}$ strains. The numbers in the first column correspond to those attached to the spots of chromatograms in the figure.

	Strains			
	Parent	R_{1b}	R_{4b}	$R_{1b(0)}$ & $R_{4b(0)}$
1. Aspartic acid	+	+	+	+
2. Glutamic acid	+++	+++	+++	+++
3. Serine	+	+	±	+
4. Glycine	++	+	±	++
5. Alanine	+++	+++	+++	+++
6. Threonine	+	+	+	+
7. Histidine	±	±	±	±
8. Tyrosine	±	+	+	±
9. Glutamine	+	+	+ ?	+
10. Proline	+	+	++	+
11. Arginine	+	+	+± ?	+
12. Methionine sulfoxide	+	++	+	+
13. Phenylalanine & norleucine	++	++	++	++
14. Leucine & isoleucine	++	++	++	++
15. Unidentifiable	+	+	+++	+

Phenylalanine and leucine did not separate respectively from norleucine and isoleucine by the solvent system used. The analyses of methionine and cystine were not tried. But methionine sulfoxide was detected, which seemed to be produced from methionine during the running with phenol[11]. Glutathione could not be determined, since its area was covered by an unidentifiable substance.

The results found are as follows. 1) Glycine and serine were almost lost in the cells of R_{4b} cultured in 4 mM Cu-MH, and the level of glycine was low in the cells of R_{1b} cultured in 1 mM Cu-MH, while the relative amounts of the other identified amino acids did not change in either of the cases. 2) Chromatograms of $R_{1b(0)}$ and $R_{4b(0)}$, which had been cultured in the normal MH, did not differ practically from that of the parent strain in the normal medium. 3) With R_{4b}, an unidentifiable spot below alanine grew so large as to make impossible the detection of arginine, glutamine, histidine, lysine and others. This spot seemed to correspond to the tripeptides identified by Dent[11, 12], since it disappeared when the extract was hydrolysed with 8 N HCl for 8 hours at 100°C in closed tube before the chromatographic development.

Discussion

The composition of amino acid pool of resistant cells was found not to differ much from that of the parent strain grown in the normal medium. It will be reported shortly that, when the parent strain is inoculated in a copper-containing medium, the amino acid pool is much disturbed for a period, recovering as the growth

of resistant cells occurs later. This implies that the growth inhibition, and then the growth, in a copper medium are accompanied by a disturbance and a recovery, respectively, of the amino acid pool, and hence of the nitrogen metabolism. However, as described earlier, even the copper-trained strains contain disturbed amino acid pools in the copper media in which their growth rates are not very much reduced. The disturbance is larger in the medium containing 4 mM of copper than in the one containing 1 mM, corresponding to the fact that the growth is less rapid in the former case. The cells produced are smaller in average size, and disjoin less easily from their parent cells, in the former case than in the latter.

The decrease in glycine and serine may be due either to an enhanced used of these amino acids or to a decrease in the production of them. However, incorporation of these amino acids into non-extractable forms did not seem to be increased, so far as the HCl-hydrolysates of extraction residues of parent cells and R$_{4b}$ were compared with each other with paper chromatography. The growth rate, i.e. the rate of synthesis of protoplasm, of R$_{4b}$ in the copper medium is somewhat lower than the parent strain in the normal medium. In spite of this, the total extractable nitrogen was lower with the former. This may favour the view that the synthesis of amino acids in general are inhibited by copper. However, an increase in substances which are suspected to be peptides may suggest that copper disturbs the nitrogen metabolism also at a level higher than amino acid synthesis.

Summary

1. The cells, trained to and grown in the medium containing 4 mM of copper, contained in their amino acid pool less of glycine and serine and more of peptide-like matter than those grown in the unpoisoned medium.

2. Chromatograms of the extracts from copper-trained substrains did not differ from that of the parent strain when they were cultured in the unpoisoned medium.

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The Effects of Phosphates on Mitosis

by Seiji Takatori*

鷹取晟二： 核分裂に対する燐酸塩の影響

Effects of various chemical and physical agents on mitosis have been reported by many workers (cf. Politzer, 1934 *et al.*). Recently Huskins (1948) and others have reported that both nucleates and phosphates greatly influence on somatic mitosis and give characteristic figures different from those of other mitotic poisons. Huskins (1948) has expressed the view that reduction divisions named "somatic meiosis" are induced in *Allium* root tips by the effect of sodium ribose nucleate and phosphates. According to other literatures on the effects of nucleates, Wilson and Cheng (1949)have supported the view of Huskins but Kodani (1948), Shimamura and Ishikawa (1951) and Woll (1953) have denied the above mentioned occurrence. Galinsky (1949) has shown that phosphates induce numeri cal reduction of chromosomes in *Allium*.

The present study has been attempted for the purpose of investigating the effects of phosphate solutions on mitoses, particularly paying attention to the "somatic meiosis".

Material and Method

Bulbs of *Allium cepa* were placed on beakers with their bases immersed in tap water. When their roots grew to a length of 2–3 cm they were placed on other beakers filled with phosphate solutions in which the roots and the bases of the bulbs were immersed. After the roots were treated with these solutions at various durations of time, several roots of each bulb were cut off, and the rest left attached to the bulb were returned to tap water. Some bulbs, on the other hand, were cultured in tap water as control. These excised root tips were immediately fixed with Navashin's fluid for 20–24 hours. Sections were made 16 μ thick and stained with Heidenhain's iron-alum-haematoxylin.

The phosphates used were NaH_2PO_4, Na_2HPO_4 and K_2HPO_4, and the concentrations of each phosphate were 0.0025, 0.005, 0.01, 0.025 and 0.05 mol. Duration of each treatment was 2, 4, 8, 12 and 24 hours. Experiment was carried out in an incubator at 25°C. The pH of solutions, distilled water and tap water was as follows:

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* Biological Laboratory, North College, Osaka University 大阪大学北校生物学研究室

NaH$_2$PO$_4$　　　　0.0025 mol……pH 4.9, 0.05 mol……pH 4.4,

Na$_2$HPO$_4$　　　　0.0025 mol……pH 8.5, 0.05 mol……pH 9.0,

K$_2$HPO$_4$　　　　0.0025 mol……pH 8.9, 0.05 mol……pH 8.9,

distilled water……pH 5.4,　　　　tap water……pH 6.8

Results

a. Cytological observation.

The first externally visible effect of the phosphate treatments was suppression of growth of the roots in the case of high salt concentrations. In this case, it was observed that the higher the concentration of the solution and the longer the duration of the treatment, the greater the grade of suppression of growth. In extreme cases, some of the roots showed a little or little growth, and in some cases root tips or whole roots were softened and killed. Even in the former cases, the roots showed marked retardation of growth when they were replaced in tap water.

In respect of the structure of nuclei in early and mid prophase there seems to be little difference between the meristem of growth-suppressed roots by the treatment and the normal untreated roots. In the later stages of mitosis, however, there are conspicuous differences between them, in respect of the nuclear structure and chromosome behavior.

Important abnormalities found in the treated roots are as follows:

(i) Chromosomes in late prophase are thick (Compare Fig. 2 with Fig. 1). (ii) In metaphase some chromosomes are more or less short and thick (Compare Fig. 4 with Fig. 3). (iii) Some chromosomes, in metaphase, take an X-shape; that is, two
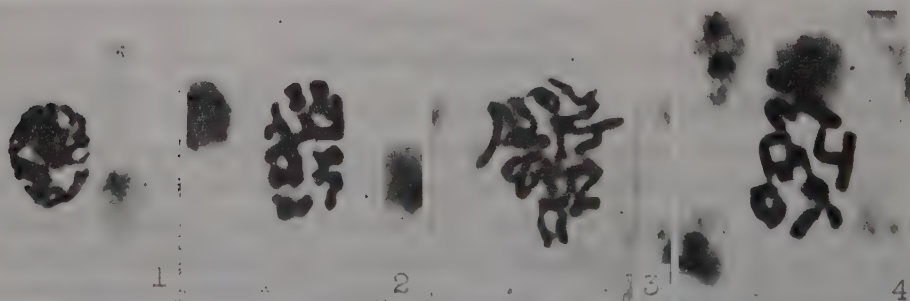
Fig. 1. Chromosomes in late prophase in an untreated root. 2. Chromosomes in late prophase in a treated root (K$_2$HPO$_4$—0.01 mol—24 hrs.). 3. Metaphase chromosomes in an untreated root. 4. Metaphase chromosomes in a treated root (Na$_2$HPO$_4$ —0.01 mol—12 hrs.). All figures represent the chromosomes found in the peripheral layers of periblem. Magnification ca 1000×.

sister chromosomes are attached at the kinetochore with four arms separate (Fig. 5). This configuration has been called by Levan (1938) as " c-pair ". It is very probable to assume that this configuration is caused by retardation of the kinetochore splitting, considering from the result of the investigation given in (b). (iv)

Rarely, chromosomes in metaphase, however, separated into two groups (Fig. 6). In Fig. 6, for example, 9 chromosomes are found in the upper group; and 7, in the lower. (v) Sometimes it is observed that one or more chromosomes are apart from

Figs. 5-7. Metaphase in treated roots.

5. X-shaped chromosomes (NaH_2PO_4—0.025 mol—4 hrs.). 6. Groupings of chromosomes into 9-7 groups (Na_2HPO_4—0.005 mol—24 hrs.). 7. Some chromosomes being apart from the others which are on the equatorial plate (Na_2HPO_4—0.005 mol—24 hrs.). Magnification *ca* 1000×.

the others which are on the equatorial plate in metaphase (Fig. 7). (vi) Frequently, anaphase chromosomes are scattered over the cell (Fig. 8). (vii) Frequently, one or a few daughter chromosomes are lagging on the equatorial plate while the others
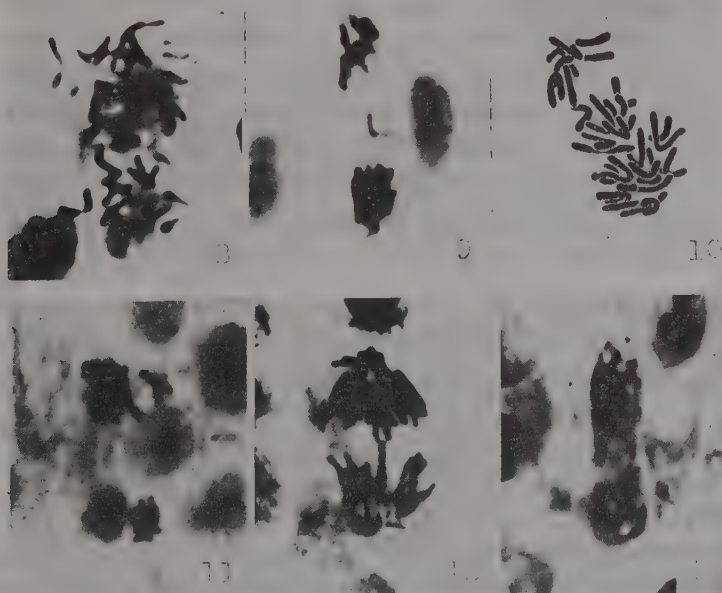
Figs. 8-13. Abnormalities in anaphase and telophase in treated roots.

8. Anaphase chromosomes scattered over the cell (Na_2HPO_4—0.01 mol—8 hrs.). 9. A few lagging chromosomes in anaphase (NaH_2PO_4—0.025 mol—4 hrs.). 10. Separation of chromosomes into approximately 7-25 groups (Na_2HPO_4—0.025 mol—4 hrs.). 11. A tetrapolar distribution (Na_2HPO_4—0.01 mol—12 hrs.). 12. A chromosome bridge in anaphase (Na_2HPO_4—0.005 mol—24 hrs.). 13. Two bridges and unequal daughter nuclei in telophase (K_2HPO_4—0.01 mol—8 hrs.). Magnification *ca* 1000×.

have already arrived at the poles (Fig. 9). (viii) Sometimes, anaphase distribution is unequal in numbers; for example, the distribution shown in Fig. 10 is approximately 7–25. (ix) Sometimes, polypolar distribution of chromosomes in anaphase takes place (Fig. 11). (x) Pycnosis of chromosomes takes place frequently. For example, one or more chromosomes bridges are formed by sticking of free ends of the sister chromosomes (Fig. 12). These bridges are sometimes found in telophase (Fig. 13). (xi) In telophase two daughter nuclei with unequal size are frequently found (Fig. 13). These nuclei seem to be formed as a result of the unequal distribution of anaphase chromosomes.

Huskins (1948) has reported that pairing of homologous chromosomes followed by their segregation, takes place in *Allium* root tips treated with sodium nucleate. In spite of the careful observation, however, neither pairing nor segregation of homologous chromosomes was found in the present investigation.

b. Frequency of mitotic stages found in root tips.

To know the effects of phosphates on progress of nuclear division in root tips, frequencies of resting stage and each stage of mitosis were measured. The results obtained are shown in Table I.

Table I. Frequency of each stage of mitoses*

A: Ratio of resting and mitotic cells in %.
B: Ratio of cells at each stage in %.

Treatment**	A		B					
	Resting stage	Mitotic stage	Early prophase	Mid prophase	Late prophase	Metaphase nor. / abnor.	Anaphase nor. / abnor.	Telophase nor. / abnor.
Control	73.5	26.5	41.7	37.8	5.3	3.5 0 / 3.5	2.5 0 / 2.5	9.2 0 / 9.2
0.05 mol NaH₂PO₄ 2 hrs.	72.7	27.3	40.3	33.7	9.0	4.1 2.8 / 6.9	2.1 0.3 / 2.4	7.6 0.1 / 7.7
0.01 mol Na₂HPO₄ 12 hrs.	82.9	17.1	37.8	36.1	7.7	3.3 4.9 / 8.2	1.6 1.9 / 3.5	6.7 0 / 6.7
0.01 mol K₂HPO₄ 4 hrs.	88.9	11.1	36.0	34.4	10.2	5.8 1.2 / 7.0	3.1 0.5 / 3.6	8.8 0 / 8.8

* Percentages were calculated on the basis of more than 1000 mitotic cells for each treatment.
** 3 or 4 roots from one bulb were used in each treatment.

From this table the following conclusions are obtained: (1) There is little difference between the percentage of the dividing cells in the root tips treated with a NaH_2PO_4 (0.05 mol—2 hrs.) solution and that of the untreated root tips. In the root tips treated with Na_2HPO_4 (0.01 mol—12 hrs.) and K_2HPO_4 (0.01 mol—4 hrs.) solutions, the frequency of mitosis is somewhat lower than that of the control.

(2) The percentages of mitotic figures in late prophase and metaphase in the treated root tips are about twice as large as those found in the untreated roots. The cause of this difference is not cleared in the present investigation, but it is highly probable that the high frequency of metaphase is caused by the delay of anaphasic polar separation, which may be due to the retardation of the kinetochore splitting.

Discussion and Conclusion

As has been described in the preceding pages of this paper, three phosphates used in this experiment disturb not only the behavior of mitotic chromosomes but the progress of mitosis.

The effects of these salts on behavior of chromosomes were essentially similar to one another. Some of the mitotic abnormalities induced by these salts seem to resemble those induced by other mitotic poisons such as nucleate, colchicine, etc. and by physical agents such as abnormal temperatures, etc.. The abnormality which was regarded as "somatic meiosis" emphasized by Huskins (1948), was not observed in the present study.

Huskins has found mitoses accompanying pairing and segregation of homologous chromosomes, followed by numerical reduction of chromosomes in *Allium* root tips treated with sodium nucleate solution, and has called these abnormal mitoses as "somatic meioses". Moreover, he has stated that the same phenomena are induced by phosphates also. Both Galinsky (1949) and Kato (1950) have treated the root tips with phosphate solutions and have observed the polar separation of un-divided chromosomes resulting their reduction in number. It has also been reported by Kodani (1948), Wilson and Cheng (1949), Shimamura and Ishikawa (1951), Woll (1953) and others that nucleates induce the same phenomenon, but Shimamura and Ishikawa have expressed the view that this phenomenon can not be regarded as "somatic meiosis".

The distribution of 16 undivided chromosomes in metaphase into two groups found in the present study is not regarded as polar separation, but as a random grouping. This phenomenon may not be regarded as chromosome segregation. It may be concluded, therefore, that what Huskins calls "somatic meiosis" was not found so far as the present experiment was concerned.

Summary

In *Allium* roots treated with NaH_2PO_4, Na_2HPO_4 and K_2HPO_4, a number of mitotic abnormalities were observed, but neither pairing nor segregation of homo-logous chromosomes was observed. The fact obtained in this experiment that the mitotic figures in metaphase are more frequently observed in the treated roots than those in the control root, may be due to the retardation of the kinetochore splitting.

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Studies on the Lotus-Seed

I.　On the Water Content of the Old but Still Viable Seed

by Yoshinobu Osawa, Chiyo Saito, and Masaru Chujo*

大沢義信・斎藤千代・中条勝：　蓮の種子に関する研究
I. 古いが尚生きている種子の含水量について

The lotus-seed retains the vital force for some thousand years if it is buried in the peart-bed**.

It has been well known that the seed could be stored very much longer under the dried condition.

The water content of the lotus-seed is very interesting in this point of view.

This report is mainly concerned with the moisture content of the lotus-seed.

Materials and Methods

The lotus-(*Nelumbo mucifera* Gartnr.) seed used in this study was collected from the bottommud of Shinobazu-pond in Tokyo, Japan, during the period from April to May 1946, and it was considered that the seed ripened more than ten years before the time collected. After collecting, that was placed in the atomospheric air at the room temperature until the measurements were performed (from Sep. to Dec. 1954), but it seems that the water content of the seed might not undergo the rapid and noteworthy change, for the seed is hard and impermeablly coated.

The weight of seed was 0.6—0.9 gram at the time determined.

The morphological characteristics did not differ from the seed harvested at present, and the ability of germination was fully maintained.

* Research Institute, Japan Anti-Tuberculosis Association

** Ohga, I. A study of the ancient but still viable fruit of the indian lotus found in the peat bed near Pulantien, South Manchuria. (1927)

The measurements of moisture content were made by the vacuum-oven method* (heating in a vacuum-oven with P_4O_{10} at about 60°C and 5 mm Hg for 3 hours), and Karl Fischer's back titration method[1]. In the latter case, the apparatus modified by the writers was used (see Fig. 1).
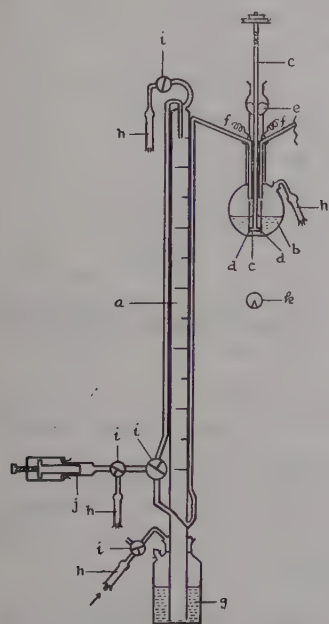
Fig. 1. The apparatus used in this study. The right half is omitted.
a; burette, b; titration flask, c; mixer, d; electrode, e; mercuric stopper, f; to the amprifier for electric titraiton, g; reagent, h; CaCl₂, i; cock, j; micro-syringe, k; magic eye.

Karl Fischer reagent used in this study was prepared by Nipponkasei Chemical Industries, Limited, Japan, and its potency was 1.83 mg./cc. The methanol containing 2 mg./cc of water, which was prepared by Kishida Chemical Co. Ltd. Japan, was used for the back titration.

The adequate number drawn from 83 seeds by the random sampling was used for testing.

After the removing of seed-coat in the dry chamber (moisture: less than 30%), the seed was divided, then the embryo and the endosperm were crushed separately using the cutter made of the bundle of the blades of safty-razor.

The weighing of the powdered sample for Karl Fischer's method was carried out in the dried place (moisture: less than 30%) on the hanging drop slide of which both wings had been cut off by one-third, and brought into the titration flask together with that glass.

It seems that the reagent may be diffused into the crushed sample within sixty minutes, for the green pigment** contained in the smashed embryo were decolourized within that time, so the titration was made at ninety minutes after the addition of samples to titration flask.

Results and Conclusions

The results obtained are shown in Tables 1 and 2.

The mean values gained by these two methods might be said to differ significantly from each other in one group of the endosperm in the fiducial limits at the 5% level, but not at the 1% level. There was no significant difference between both values in the other group of the endosperm and the embryo at the 5% level (see Tables 1 and 2).

The values of water content of the embryo were almost same as that of the albumen in Karl Fischer's method.

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* [The standards of biological medicaments] issued in 1953 by the Ministry of Health and Welfare, Japanese Government.

** This pigment was identified with chlorophyll a and b by these writers (unpublished).

Tab. 1. The water content of lotus-embryo determined by two methods.

Exp. No.	Weight of sample in mg.	Moisture content %	Average	Method
1	25.19	4.36		
2	17.57	4.82	4.07	
3	17.51	3.94	±0.72	
4	18.12	3.25		
5	17.59	3.98		vacuum-oven
6	19.79	4.09		method
7	17.83	3.98	3.99	
8	19.91	3.91	±0.23	
1	181.15	4.20		Karl
2	101.02	5.18	4·66	Fischer's
3	161.41	4.89	±0.72	titration
4	183.60	4.38		

Note: Two groups having the average value of 4.07 and 3.99 were measured on the other day in the entirely same manner.

Table. 2. The water content of lotus-endosperm determined by two methods.

Exp. No.	Weight of sample in mg.	Moisture content %	Average	Method
1	73.81	7.96		
2	57.51	5.27	6.78	
3	89.77	6.07	±1.60	
4	94.14	8.29		
5	78.84	6.31		vacuum-oven
6	88.29	4.68		method
7	74.96	7.40		
8	72.23	6.86	5.97	
9	110.01	6.24	±1.56	
10	72.04	4.67		
1	106.12	4.85		Karl
2	340.58	4.42	4.86	Fischer's
3	250.77	5.36	±0.61	titration
4	292.10	4.81		

Note: Two groups having the average value of 6.78 and 5.97 were measured on the other day in the entirely same manner.

On the other hand, the one mean value obtained by the vacuum-oven method with the endosperm was observed to differ significantly from that of the embryo at the 1% level and the other one differ noteworthy from that of embryo at the 5% level, but not at the 1% level.

The constant value was seldom given by the vacuum-oven method with the endosperm, i.e. its deviation was somewhat larger than in other cases, and it was probably introduced from the crushing treatment for a rather long time in this case, so the value generally showed a larger numeral than what it was.

we may believe that the values of moisture content of embryo and endosperm obtained by the vacuum-oven method are also equal, if we consider this circumstance.

Now, M. Izawa[1,2], assertained that the value obtained by the gravimetry was obviously smaller than that of Karl Fischer's method with penicillin in oil and wax and some other biological products, and deduced that the difference may be explicable as three alternatives:

1) Karl Fischer reagent may react with some compounds in the sample other than the water.
2) By the ordinary method, the water cannot be eliminated completely.
3) The nature of water detectable by the two methods may be different, that is, as Karl Fischer's method is carried out under dissolved or finely dispersed states, the water such as crystaline water should also be detectable, which cannot be removed by heating.

Then, he concluded that the water cannot be removed completely by the conditions described in his paper.

While, he demonstrated that the results of two methods in the cases of solid penicillins showed nearly the same order and the consumption of the reagent became negligible when samples were dried in the vacuum-oven for an appropriate time.

So, we may say that the moisture content of lotus-seed is determined at about the same order by the two methods as in the cases of solid penicillins and that the embryo contains the same amount of water as the endosperm.

At any rate, the content of water is not extremly small in the lotus-seed examined. In addition, I. Ohga reported in 1927 on the problem in question by using the ordinary gravimetry as follows: old fruit; 12.38%, new fruit; 12.5%.

Thus, the reason why the lotus-seed retain the vitality for a long time may not be in the smallness of water content.

Summary

1) The water content of lotus-seed collected from the bottom-mud of pond was determined by the use of two methods.
2) The apparatus for Karl Fischer's back titration method was modified by the writers, and used in this study.
3) The results obtained from the different regions of seed by these two methods were compared, and then discussed.
4) Because the moisture content of the seed is not extremly small, the reason why the lotus–seed retain the vitality for a long time may not be in the smallness of water content.

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Karyotaxonomy in Poaceae IV

Chromosomes and Systematic Relationships of Several Species.

by Tuguo TATEOKA [*]

館岡亜緒： イネ科植物の核学的分類学 IV.
数種の染色体と分類学的考察

Chromosomes were studied in root-tip cells. The materials were treated according to the same method as described in the previous reports. The sources of the materials used are given in the following table:

Table 1. The sources of materials

Species	Sources
Bromus tectorum, Melica ciliata, Briza media, Festuca ciliata, Poa nemoralis	Bot. Gart., Berlin-Dahlem, Germany.
Lolium italicum, L. rigidum	Inst. ed Orto Bot. di Roma, Itarly.
Uniola latifolia	Inst. ed Orto Bot.dell Univ.di Roma, Italy.
Cynosurus cristatus	Bot.Gart. Friedrich-Schiller Univ., Yena, Germany.
Sieglingia decumbens	Bot.Mus., Copenhagen, Denmark.
Oplismenus compositus, Setaria palmifolia, Paspalum orbiculare, Panicum repens	Anbô, Yaku Island, Kagoshima Pref., Japan.
Lophatherum sinense, Chikusichloa aquatica	Hitoyoshi, Kumamoto Pref., Japan
Setaria excurrens var. pauciseta	Setoishi, Kumamoto Pref., Japan.
Pogonatherum crinitum	Tanabe, Wakayama Pref., Japan.
Phragmites Karka	Bot.Gart. Kyoto Univ., Japan.
Asperella longe-aristata, Spodiopogon sibiricus	Hakone, Kanagawa Pref., Japan.

The seeds of all species foreign to Japan were kindly supplied by various institutions to whom the author wishes to express his cordial thanks. They were raised in our experimental garden and used in the present study. The Japanese species were through-out collected by the author, and the root-tips were fixed immediately at the habitats.

Observations

Chromosome numbers are listed in Table 2 together with previous records. Chromosome sizes are shown in Figs. A, 1~21.

[*] National Institute of Genetics, Mishima Shizuoka Pref., Japan.

Table 2.   List of chromosome numbers observed.

Species (Japanese name)	2n	Fig.(A)	Previous records 2n	Authority
Asperella longe-aristata (Hack.) Ohwi (Azumagaya)	28	9		
Bromus tectorum L.	14	1	14	Cugnac et Simonet 1941
Lolium rigidum Gaud.	14	3	14	Jenkin 1954, etc.
L. italicum A. Br.	14	2	14	Jenkin 1954, etc.
Briza media L.	14	4	14	Avdulov 1931
Cynosurus cristatus L.	14	5	14	Avdulov 1931
Festuca ciliata	14	6		
Poa nemoralis L.	35	7	28	Avdulov 1931
			42	Armstrong 1937, etc.
Uniola latifolia Michx.	48	8	48	Avdulov 1931, Brown 1950
Sieglingia decumbens (L.) Bernh.	36	11	36	Scheerer 1940
			124	Maude 1940
Melica cilita L.	18	10	18	Avdulov 1931
Phragmites Karka Trin. (Seitakayosi)	48	14		
Lophatherum sinense Rendle (Tô-sasa-kusa)	48	12		
Chikusichloa aquatica Koidzumi (Tsukusigaya)	24	13	24	Hirayoshi 1937
Oplismenus compositus Beauv. (Edauchi-chizimizasa)	72	18	72	Avdulov 1931
Setaria palmifolia Stapf (Sasakibi)	54	19		
S. excurrens Miq. var. *pauciseta* Ohwi (8o-sasakibi)	72	20		
Paspalum orbiculare Forst. (Suzumeno-kobie)	60	21		
Panicum repens L. (Haikibi)	45	17		
Pogonatherum crinitum Kunth (Itachi-gaya)	20	16		
Spodiopogon sibiricus Trin. (Ō-abura-susuki)	40	15		
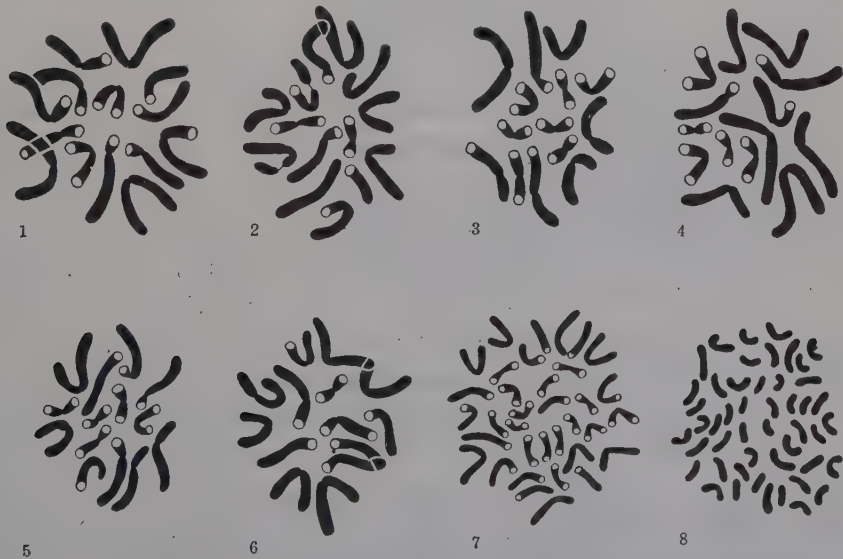
Fig. A. 1~8.   Somatic chromosomes. X 2000.   1, *Bromus tectorum.*   2, *Lolium rigidum.* 3, *L. italicum.*   4, *Briza media.*   5, *Cynosurus cristatus.*   6. *Festuca ciliata.*   7, *Poa nemoralis.*   8, *Uniola latifolia.*
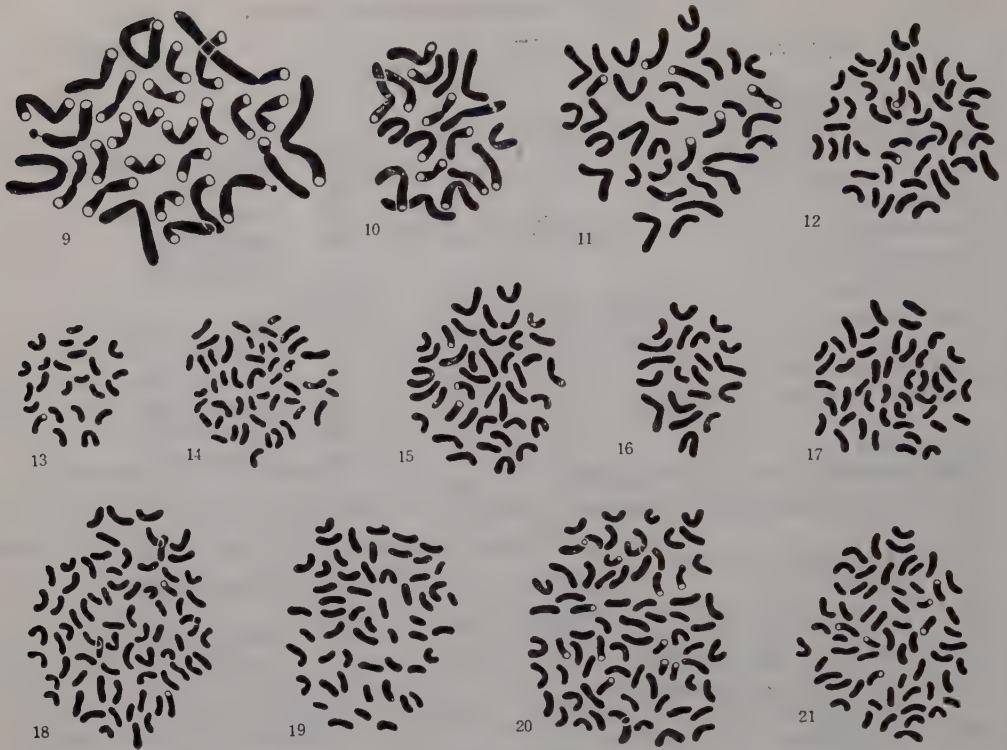
Ffg. A. 9~21.　Somatic chromosomes. X 2000　9, *Asperella longe-aristata*　10, *Melica ciliata*. 11, *Sieglingia decumbens*.　12, *Lophatherum sinense*.　13, *Chikusichloa aquatica*.　14, *Phragmites Karka*.　15, *Spodiopogon sibiricus*,　16, *Pogonatherum crinitum*.　17, *Panicum repens*.　18, *Oplismenus compositus*.　19, *Setaria palmifolia*.　20, *S. excurrens* var. *pauciseta*.　21, *Paspalum orbiculare*.

Considerations

Among species whose somatic chromosomes are reported in the present paper, *Festuca ciliata*, *Briza media*, *Cynosurus cristatus*, *Poa nemoralis* and *Uniola latifolia* belong to Festuceae-Festucinae according to Pilger's classification (1954).　While placing them together in one group appears justified by their external morphology, various kinds of basic chromosome numbers and chromosome sizes are found among them : *Festuca*, *Briza*, and *Cynosurus* :—b=7, large ; *Poa* :—b=7, medium sized ; *Uniola* :—b=12 (or 6), small (cf. Figs. A 4~8).　The differences in chromosome characteristics are associated with distinctive features of the epidermis and transverse leaf section.　A particular consideration of their systematic relationships will be given in a later paper .and the author will here confine himself to the description of the chromosome situation.

The genus *Sieglingia* had been treated as a member of Festuceae, as well as the genus *Triodia* in which *Sieglingia* was formerly included.　Hubbard (1948) believes in an affinity between *Sieglingia* and *Danthonia*, relying on the existence of natural hybrids between them, the resemblance in the structure of their spikelets and the development of cleistogenes in the axils of the basal leaves of the flowering culms.

The somatic chromosome number of *S. decumbcens* is thirty six in accord with Scheerer's (1940) observation. The basic chromosome number ot this genus may be six in agreement with that of *Danthonia*, although the number of nine may be also considered as its basic number. The size of the somatic chromosomes of the two genera is very similar. The genus *Tridens* formerly treated as a section of *Triodia*, like *Sieglingia*, is clearly different from *Sieglingia* with respect to the chromosomes and the characteristics of the epidermis and transverse leaf section. According to Brown (1951), species of *Tridens* have small chromosomes and multiple numbers of eight. The author's observations of the epidermis and transverse leaf section in both genera are as follows: *Tridens flavus*, *T. strictus*, *T.* (*Rhombolytrum*) *albescens*:— chloroplasts are localized in a characteristic cell layer directly surrounding the vascular bundles. Saddle shaped siliceous cells are found in the epidermis and bicellular hairs shaped like a cannon ball (Figs. BI, 1~3). *Sieglingia decumbens* :—chloroplasts are uniformly distributed throughout the mesophyll. Dumbbell shaped siliceous cells and threadlike bicellular hairs are found in the epidermis (Figs. BI, 4~5). The characteristics of the epidermis and transverse leaf section found in *Tridens* species are the same as in the Eragrostoideae. On the other hand, points of similarity in those characteristics are found between *Sieglingia* and some members of *Danthonia* genus (cf. de Wet 1954b). Tne relationship of *Sieglingia* and *Danthonia*, and the placing of *Tridens* in the subfamily Eragrostoideae are supported by the character of chromosomes and the properties of the epidermis and transverse leaf section.
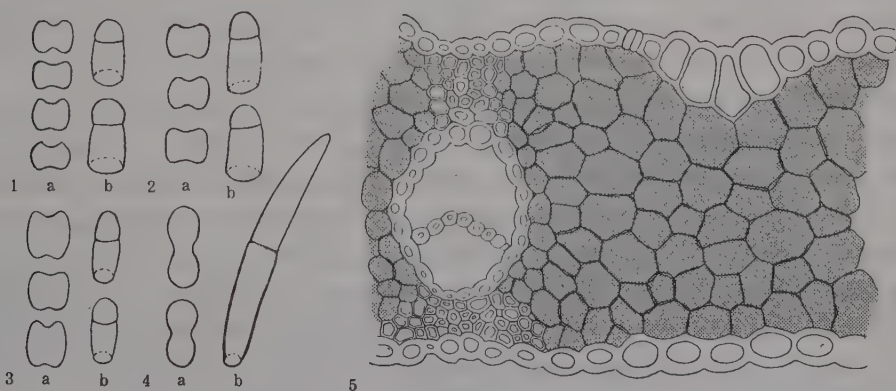
Fig. B I, 1~4. Siliceous cells and bicellular hairs found in tne lower epidermis. 1, *Tridens flavus*. 2, *T. strictus*. 3, *T. albescens*. 4, *Sieglingia decumbens*. a-Siliceous cell. b-Bicellular hair. 4b X 300, the others X 450.

Fig. B I, 5. A transverse section of the leaf of *Sieglingia decumbens*. X 200.

The genus *Chikusichloa* is usually treated as a member ef Oryzeae, whose majority of genera are characterized by small chromosomes and the basic number 12 (or 5). The chromosome complement of *Chikusichloa* clarified by Hirayoshi (1937) and reexamined in the present study is the same as that of typical members of

Oryzeae, i.e. b=12 and the chromosomes are small. The author studied the charact-
eristics of the epidermis and transverse leaf section in *C. aquatica* with the object
of further ascertaing its systematic placing. For the comparison with *Chikusichloa*,
four Japanese species belonging to *Oryza, Leersia* and *Zizania* (Oryzeae) were ex-
amined. The results obtained are as follows: *Chikusichloa aquatica*:—chloroplasts
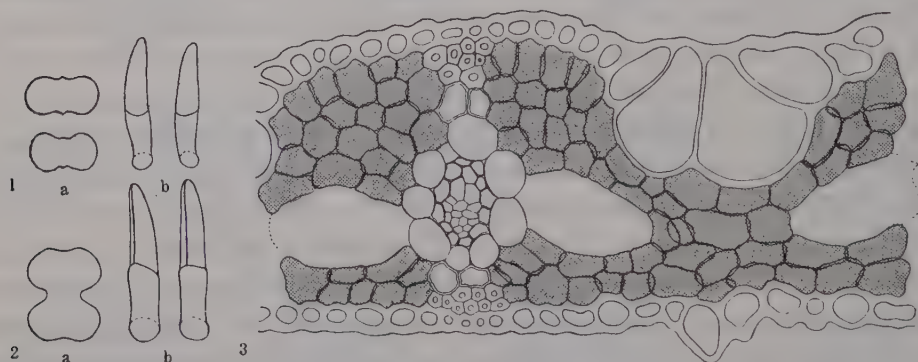
Fig. B, II. 1~2. Siliceous cells and bicellular hairs found in the epidermis. 1, *Leersia*
japonica. 2, *Chikusichloa aquatica.* a-Siliceous cell. b-Bicellular hair. X 600.
Fig. B, II. 3. A transverse leaf section of *Chikusichloa aquatica.* X 300.

are uniformly distributed throughout the mesophyll and the vascular bundles are
surrounded by a cell layer which does not contain chloroplasts. Threadlike
bicellular hairs and dumbbell shaped siliceous cells (whose longitudinal axis coincides
with that of the blade) are found in the epidermis (Figs. BII, 2~3). *Oryza sativa,*
Leersia japonica, L. oryzoides var. *Sayanuka* and *Zizania latifolia*:—chloroplasts are
uuiformly distributed throughout the mesophyll and a cell layer without choroplasts
is found surrounding directly the vascular bundles. The epidermal characteristics of
the four species are in agreement with Part's (1936) Oryzoid subtype; bicellular
hairs are threadlike and siliceous cells are dumbbell shaped (their longitudinal axis is
perpendicular to that of the blade (Fig. B II, 1). The resemblance in the properties
of the epidermis and transverse leaf section between *Chikusichloa aquatica* and the
other species described above is considerable, and the difference lies only in the shape
ofsiliceous cells. The difference is not sufficient to justify the separation of the
genus *Chikusichloa* from Oryzeae, and *Chikusichloa* should be regarded as a member
of Oryzeae, considering the resemblance in the chromosome situation, spikelet struc-
ture and leaf-anatomy. Ohwi (1941) is of the opinion that *Chikusichloa* should be
treated as an independent subtribe of Oryzeae. His opinion is based on the distinctive
features of external morphology. Ohwi may be right, considering the facts described
above.

The magnification of figures published by de Wet (1954) was not stated. Chromosome sizes of this
species may be, however, estimated to be small, judging from their rod-like shape and from the
comparison with the chromosome figures of other species published together.

Pogonatherum and *Spodiopogon* which had not been studied cytologically up to the present belong to the tribe Andropogoneae. Their chromosome situation clarified in the present study is the same as that found in most genera of Andropogoneae: b=10 (or 5) and the chromosomes are small. While Andropogoneae represent a definite group with resepct to external and internal morphology, some differences in the chromosomes are found between the genera and even within a single genus, *Hemarthria*. Brown (1951) reported 2n=20 large chromosomes for *Elyonurus trip-sacoides* and *E. barbiculmis*, and also 2n=18 medium sized chromosomes for *Manisu-ris cylindrica*. In contrast with those three species, a great majority of genera in Andropogoneae are characterized by small chromosomes similar to those of *Spodio-pogon* and *Pogonatherum* mentioned above. Ono and Tateoka (1953) reported 2n= 18 medium sized chromcsomes for *Hemarthria sibirica*, whereas de Wet (1954a) found 2n=20 small chromosomes in *H. altissima*. The chromosome situation of *H. sibirica* is the same as in *Manisuris cylindrica*, indicating a phylogenetic relationship. However, the species which have been cytologically studied are too few to be submitted to discussion regarding their systematic relationships.

The basic chromosome number of *Lophatherum* reported for the first time in the prrsent paper is 12 (or 6) and they are small, in agreement with the related genus *Centotheca*. Species of *Asperella, Oplismenus, Setaria, Paspalum* and *Phrag-mites* whose somatic chromosomes were examined (Table 2) reveal the same chro-mosome situation as that of tribes to which each species refers: *Asperella* (Triti-ceae)—b=7, large; *Oplismenus, Setaria, Panicum, Paspalum* (Paniceae)—b=9 or 10, small; *Phragmites* (Arundineae)—b=12 (or 6), small.

Summary

Somatic chromosomes of twenty one grass species were examined. The systema-tic placing of *Sieglingia, Tridens* and *Chikusichloa* was discussed on the basis of distinctive features of the epidermis, transverse leaf section and chromosome situa-tion. Diversity of chromosomes concerning their basic number and size in Festuceae-Festucinae and Andropogoneae was pointed out. The character of the chromosome complements of the other species reported in this paper is in agreement with that of the tribes to which they belong.

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Study on the Vertical Distribution of Epiphytic Bryophytes on Beech Trees, *Fagus crenata* Blume[*]

by Yoshiwo HORIKAWA & Satoshi NAKANISHI[**]

堀川芳雄・中西哲：　ブナに着生する蘚苔類の垂直分布について

The epiphytes in the coniferous, evergreen and deciduous forests in the moun-tain area of Japan have a wide range of development and grow in considerable abundance. This is especially true of bryophytes and lichens. Ecological studies of epiphytic communities in the forests of Japan have been incomplete, because there are many unavoidable difficulties in collecting the data in the field works.

In both Europe and North America, attention has been attracted to the subject of epiphytic bryophyte communites to a considerable extent but only a few reports on their distribution on the supporting-tree have been published.[4,6] Recently, M. E. Hale[2] reported that "the cryptogams have a definite pattern of distribution along the trunk with each species reaching a maximum value of constancy at one level".

When the above information on the vertical distribution of epiphytes is con-sidered from a synecological standpoint, it may be understood that the floristic composition of the epiphytic community is not uniform but different in each part of the supporting-tree, e. g. the trunk base, the trunk, the crown base and the crown.

In the present paper, the writers report the results obtained from a study of the vertical distribution of epihytic bryophytes on the trunks of *Fagus crenata* Blume in the two different places, namely the mounain-ridge and the mountain-side places[***], of Mt. Kammuri in the western part Hiroshima Prefecture.

General View of the Two Places

This study was made during the summer of 1953 on ten beech trees in the mountain-ridge at an elevation of about 1320m above sea level and on six trees in the mountain-side at an elevation of 1140 m above sea level, which is considered to

be the lower limit of the beech forest in this district.

The averge height of investigated trees is about 15.5 m (the minimum is 13.5 m and the maximum 19 m) in the mountain-ridge, while in the mountain-side it is about 17 m (the minimum is 14 m and the maximum 20 m). The average value of D.B.H. (Diameter Breast Height) is 0.27 m and 0.32 m in the mountain-ridge and the mountain-side respectively. Branching of the boughs begins at a height of 6m in the former and 6.5 m in the latter.

The forest community in the mountain-ridge is generally composed of the following specices: in the arboresent layer, *Fagus crenata* BLUME, *Acer sieboldianum* MIQUEL; in the fruticose layer, *Lindera umbellata* THUNB., *Symplocos chinensis* (LOUR). DRUCE.; in the herbaceous layer, *Carex foliossima* FR. SCHM., *C. multifolia* OHWI. On the other hand, in mountain-side, *Fagus crenata* BLUME aud *Quercus mongolica* var. *grosseserrata* (BLUME) REHD- et WILS. are dominant in the arborescent layer, a:d *Sasa veitchti* (CARR.) REHD. is the characteristic species of the herbaceous layer, while in the frutiose layer there is no dominant species at all.

Methods and Treatment of Data

When the sample trees were selected for the purpose of our study the necessary considerations were paid to select them lest some trees in a small limited area should be the reprsentative trees of the whole area. Then, the trees were selected from several plots in the whole area.

And the studies were confined to living trees having no liana on their trunks and those with more straight trunks.

The epiphytic community on each supporting-trees is sampled by the following five cylindrical quadrats: from the ground to 0.5 m; 2.5 m to 3.5 m; 5.5 m to 6.5 m; 8.5 m to 9.5 m; and 11.5 m to 12.5 m, and the names of the bryophytes occuring in each quadrat were recorded on data sheets. The vertical extent of each cylindrical quadrat is one meter except the trunk base and, although the area of each quadrat varies according to the diameter of the trunk, the number of species in a quadrat seems not to be governed by the variation of the area's size.

The following coincidental relationship of the situation is recognized between the **international** quadrats and the parts of the supporting-tree.

Quadrats	Parts of the supporting tree
From the ground to 0.5 m	Trunk base
2.5– 3.5 m	Trunk
5.5– 6.5 m	Upper trunk or Grown base
8.5– 9.5 m	Crown
11.5–12.5 m	Upper crown

The occurrence of bryophytes on all investigated supporting-trees, regardless of each quadrat, is best characterized as Presence, by assigning the rank of " stand "

to a single investigated tree. The data obtained from quadrats at the same level are here given as Constancy, although the sample areas vary in the total size but not in the vertical extent. And the degree of vertical distribution of every species, obtained from the average value of the frequency which is decided by dividing the number of occurred quadrats by the total number of quadrats in a supporting-tree, is represented by the following five indexes: I occurring below 20%, II from 21% to 40%, III from 41% to 60%, IV from 61%, to 80%, of average frequency.

Results

1. The state of the vertical distribution of epiphytic bryophytes in the mountain-ridge.

Of 41 species of epiphytic bryophytes counted in the mountain-ridge, 32 species are mosses and the rest hepatics. The presence, constancy and the degree of vertical distribution of the major species are given in Table 1. In the mountain-ridge, *Boulaya mittenii*, *Frullania moniliata* subsp. *obscura* and *Macrosporiella dozyoides*, as a whole, are dominant and the physiognomy of epiphytic communities in the beech forest is characterized by the growth-forms of these species.

According to the value of constancy on different levels, the species can be divided into four distributional types, though they are not construed as rigid categories. The state of the vertical distribution of typical species belonging to each distributional type is illustrated in Fig. 1.

The occurrence of the first type is generally restricted to the trunk base level
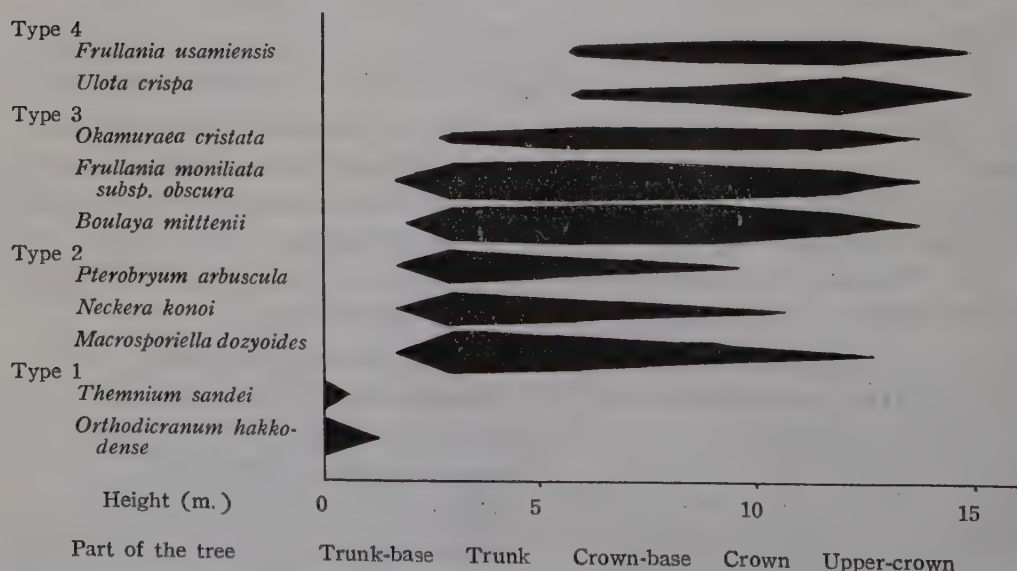
Fig. 1. Diagram showing the state of the vertical distribution of typical species belonging to respective distributional types.

Table 1.   Major species of epiphytic bryophytes occurring on beech trees
in the mountain-ridge.

Growth forms	Species	Presence %	Constancy % Quadrats					Degree of vertical distribution
			0 ~ 0.5m	2.5 ~ 3.5m	5.5 ~ 6.5m	8.5 ~ 9.5m	11.5 ~ 12.5m	
C	Orthodicranum hakkodense	80	80	—	—	—	—	I
FS	Anomodon ferrugineus	80	80	—	—	—	—	I
D	Thamnium Sandei	50	50	—	—	—	—	I
D	Dolichomitriopsis diversiformis	40	40	—	—	—	—	I
Sf	Homalia joponica	40	40	—	—	—	—	I
M	Plagiothecium denticulatum	40	40	—	—	—	—	I
Sf	Fissidens gymnogynus	30	30	—	—	—	—	I
Sf	Plagiochila ovalifolia	30	30	—	—	—	—	I
M	Brachythecium plumosum	30	30	—	—	—	—	I
Lp	Metzgeria furcata	30	30	—	—	—	—	I
Fs	Macrosporiella dozyoides	100	—	100	80	40	10	III
Bf	Neckera konoi	90	—	80	50	20	—	II
Bf	Pterobryum arbuscula	80	—	80	40	10	—	II
Fs	Anomodon giraldii	70	40	50	30	10	—	II
Hp	Radula sp. (complanata ?)	60	—	20	40	—	—	I
Tp	Dicranum fauriei	50	—	50	30	10	20	II
Hp	Madotheca vernicosa	40	10	30	—	—	—	I
Fs	Miyabea furticella	40	—	30	30	—	—	I
Bf	Neckera yezoana	30	10	20	—	—	—	I
D	Dolichomitra cymbifolia	20	—	20	20	—	—	I
Hp	Madotheca ulophylla	20	—	10	—	10	—	I
Bf	Bissetia lingulata	20	—	20	20	—	—	I
Fs	Boulaya mittenii	100	—	80	90	90	60	IV
Hp	Frullania moniliata subsp. obscura	100	—	80	100	80	50	III
Fs	Okamuraea cristata	90	—	20	50	50	40	III
Hp	Frullania japonica	60	—	30	10	30	20	I
Fs	Macrosporiella scabriseta	50	—	10	30	40	20	II
cp	Ulota crispa	90	—	—	20	40	80	I
Hp	Frullania usamiensis	90	—	—	20	50	60	I
Fs	Macromitrium brachycladulum	60	—	—	10	20	30	I

where the humus layer shows a considerable development and little change of
climatic factors is considered. Species of this group are *Orthodicranum hakkodense*,
Anomodon ferrugineus, *Thamnium Sandei*, *Dolichomitriopsis diversiformis*, *Homalia
japonica*, etc. The second type is the bryophytes which show the greatest value of
constancy in the part between 2.5 m and 3.5 m in height, where these species find
the fitful condition for their thriving, and their value of constancy gradually becames
smaller in either upper parts or lower parts. *Macrosporiella dozyoides*, *Neckera
konoi*, *Pterobryum arbuscula* and *Anomodon giraldii* are typical species of this group.

The type 3 includes the bryophytes which occur with the maximum value of con-
stancy in the part between the upper trunk and the crown and still show a relatively
high constancy even in the upper crown but not occur in the trunk base. *Macrosporiella
scabriseta, Frullania moniliata* subsp. *obscura, Boulaya mittenii* and *Okamuraea
cristata* belong to this group. The members of the last type show the greatest value
of their eonstancy in the upper crown, and are usually absent below the trunk.
They are *Ulota crispa, Macromitrium braccycladulum* and *Frullania usamiesis*.

　　2. The state of the vertical distribution of epiphytic bryophytes in the
　　　　mountain-side.

In the mountain-side, 12 species of mosses and 2 species of hepatics were found.
This number is about one third of the total number of species in the mountain-
ridge. The presence, constancy and the degree of vertical distribution of each
species are shown in Table 2.

Table 2.　Species of epiphytic bryophytes occurring on beech trees in
the mountain-side.

| Growth forms | Species | Presence % | Constancy % | | | | | Degree of vertical distrifbution |
| | | | Quadrats | | | | | |
			0 ~ 0.5m	2.5 ~ 3.5m	5.5 ~ 6.5m	8.5 ~ 9.5m	11.5 ~ 12.5m	
D	*Dolichomitriopsis diversifomis*	83	83	—	—	—	—	I
M	*Brachythecium plumosum*	50	50	—	—	—	—	I
Fs	*Anomodon giraldii*	33	33	—	—	—	—	I
M	*Thuidium cymbifolium*	17	17	—	—	—	—	I
Bf	*Neckera yezoara*	17	17	17	—	—	—	II
Hp	*Frullania usamiensis*	67	—	33	50	17	—	I
Fs	*Okamuraea crtstata*	50	—	50	33	—	—	I
Fs	*Macrosporiella dozyoides*	33	—	33	17	—	—	II
Fs	*Macrosporiell scabriseta*	33	—	33	—	—	—	I
Hp	*Frullania moniliata subsp. obscura*	33	—	33	—	—	—	I
Fs	*Boulaya mittenii*	33	—	33	17	—	—	I
Bf	*Neckera konoi*	17	—	17	17	—	—	II
Fs	*Anomodon abbreviatus*	17	—	17	—	—	—	I
cp	*Ulota crispa*	100	17	67	67	33	17	II

In the place, it is clear that the vertical distribution of every species is more
narrowly limited than in the mountain-ridge. And it is also obvious that the
situation where the species, which can be found in both places, grow in the best
condition is lower in the mountain-side than in the ridge. It must be noticed with
an interest that *Ulota crispa* growing in the maximum constacy in the upper crown
part of the supporting-trees in the mountain-ridge, appears, in the mountain-side,
in all parts of the supporting-trees, though its constancy is smaller.

　As mentioned above, there are several obvious differences in the growing state

of the vertical distribution between the mountain-ridge and the mountain-side. Consequently, the distributional pattern which was observed in the mountain-ridge seems to be not always the same to that of the mountain-side; that is, one is inapplicable to the other. It may be more appropriate to divide the species in the mountain-side into two groups: one whose maximum constancy is at the trunk part, and the other at the trunk base.

　　3. A comparative consideration of the vertical distribution in two places viewed from the stand point of growth-form.

　　The relative number (%) and the average constancy of species belonging to each growth-form type of epiphytes[3] are obtained with respect to every parts of the supporting-trees in two places, and the resultare shown in Fig. 2. And the spectrum of growth-forms in both places is shown in Table. 3.
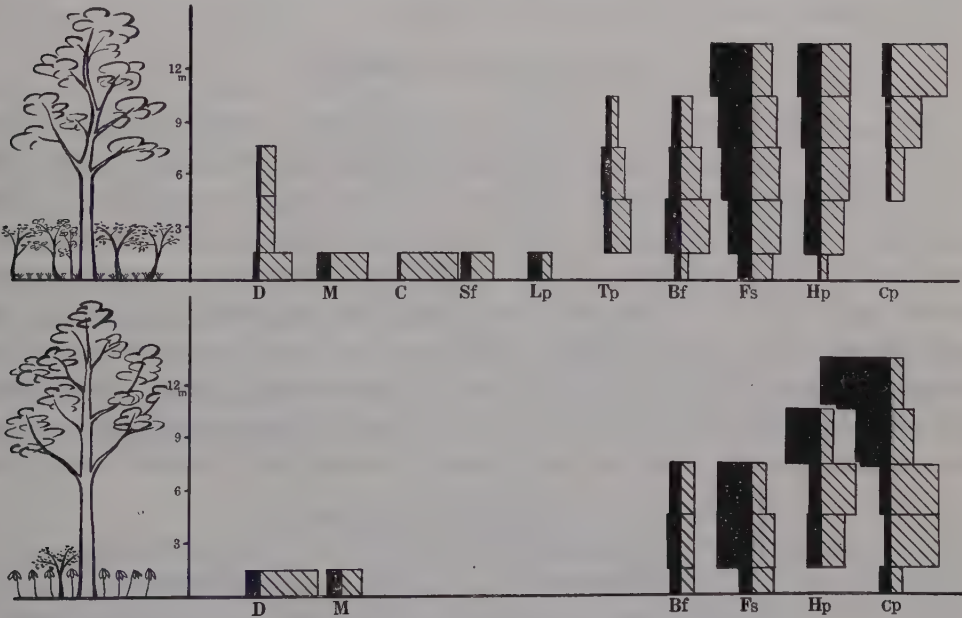
Fig. 2. This growth-from spectrum of epiphytic bryophytes in each part of the supporting-tree. The black portion indicates the relative number (%). and the portion with oblique line indicates the average constancy.

Table 3. The growth-form spectra of epiphytic bryophytes in two places.

Growth forms	D	M	C	Sf	Ef	Lp	Tp	Fs	Hp	cp
Mountain-ridge	7	10	2	7	12	10	5	29	15	2
Mountain-side	7	14	—	—	14	—	—	42	14	7

　　The growth-forms such as *carpet* type (**C**), *simple feather* (**Sf**), *loosely pressed mat* type (**Lp**) and *turf* type (**Tp**), which occur in the mountain-ridge, are absent in

the mountain-side. And the growth-form of *small cushion* type (**cp**), which is characteristic of the upper crown part in the mountain-ridge, has a wide vertical distribution in the mountain-side. The growth-form of *fascicular* and *shrubby* type (**Fs**) and *hardly pressed mat* type (**Hp**) are found in all parts of the supporting trees in the mountain-ridge, but are rather limited to the lower part of the trunk in the mountain-side.

The stems of plants classified into the growth-form of *small cushion* type (**cp**) are in close contact with each other and form the " moss ball " in appearance. It may be considered that this growth-form is more familiar to a dry condition than other growth-types. It may be considered that, contrary to the case in the mountain-ridge, the contrast been the vigorous growth of *small cushion* type (**cp**) and the dull growth of *fasicular* and *shrubby* type (**Fs**) and *hardly pressed mat* type (**Hp**) in the mountain-side is due to the dryness in there, which is immoderate to some extent, compared with that in the mountain-ridge.

Discussion and Conclusion

From our observations, it becomes clear that each species does not occur all over the supporting-tree but its occurrence is considerably confined to limited parts of the supporting-tree, in other words, each species has a definite pattern of the distribution which is peculiar to it. Thus, it is recognized that the epiphytic bryophyte community has a substantial tendency, which is noteworthy, to occur in zonal arranagement. The fact mentioned above shows that the respective parts of the supporting-tree have peculiar habitat conditions for the bryophytes to thrive. Therefore, it seems reasonable to say that it is an essential, indispensable method for the solution of ecological studies of epiphytic communities to divide the supporting-tree into several parts, as was already proposed by Ochsner[5] and after-

Table 4.　The zonal arrangement in two places.

Part of the tree	Height	Mountain-ridge		Mountain-side	
		Main growth-forms	Major species	Main growtn-forms	Major species
upper crown	above 10m	cp Hp	*Ulota crispa* *Frullania usamiensis*	cp	*Ulota crispa* (rare)
crown base	5.5~9m	Hp Fs	*Boulaya mittenii* *Okamuraea cristata* *Frullania moniliata* subsp. *obscura*	cp	*Ulota crispa*
trunk	2~5m	Bf Fs	*Macrosporiella dozyoides* *Neckera konoi* *Pterobryum arbuscula*	Fs Hp cp	*Ulota crispa* *Frullania usamiensis* *Okamuraea cristata*
trunk base	below 1m	M Fs Sf D	*Orthodicranum hakkodense* *Anomodon ferrugineus* *Thamnium sandei*	D M	*Dolichomitriopsis diversiformls* *Branchythecium plumosum*

words emphasized by Braun-Blanquet[1]

The state of zonal arrangement observed in the mountain-ridge differs from that in the mountain-side. The zonal arrangements in the two places are comparatively summarized in Table 4.

The epiphytic bryophyte community on the young beeches in the mountain-ridge is generally similar, in its floristic composition and physiognomical character, to that on the old beeches in the mountain-side. Then, it may be considered that the species found in the latter are the "pioneer" elements, as reported by E. Quarterman[7] in a early stage of the development of epiphytic community on the bark of beeches. However, judging from the fact that the average values of D. B. H. of investigated supporting-trees in the two places show little difference, the ages of beech forests in the two places are considered to be almost of the same. Therefore, such successional view point as mentioned above may be relegated to a subsidiary position of consideration for the present.

Thus, it follows probably that the notable differences of zonal arrangement between the two places may be due to the differences of climatic factors brought by the differences of the topographical conditions. The ridge of this mountain, being higher than the surrounding mountains, acts necessarily as a barrier rôle, where very moist air coming from the Sea of Japan in winter and from the Pacific Ocean (or the Setonaikai) in summer season is always kept. Consequently, the mountain-ridge of Mt. Kammuri is always kept in a comparatively damper condition than the mountain-side all the year round.

It seems to be natural to recognize that the epiphytic bryophyte communities in both places are in the stage of climax with its own peculiar composition according to the differences of climatic factors.

Summary

1. An investigation of 16 beech trees (*Fagus crenata* BLUME.) in the forest of Mt. Kammuri, located in the western part of Hiroshima Prefecture, was conducted to determine the vertical distribution of epiphytic bryophytes.

2. All species taken up in the investigation of the mountain-ridge are grouped into four distributional types: the groups of species showing their maximum flourishing (1) at the trunk base, (2) at the trunk, (3) at the upper trunk and crown base, (4) at the upper crown.

3. It recognized that the epiphytic bryophyte communities show a substantial tendency of the zonation in the vertical distribution observed in the supporting-trees.

4. There are some remarkable differences in the state of zonal arrangement between the mountain-ridge and the mountain-side. The comparative summarization of the zonal arrangement in each place is shown in Table 4.

5. It seems to be that differences in the state of zonal arrangement between

the two places are mainly due to the difference of climatic factors brought by topo-graphical conditions.

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速 報

Auxins and Free Sulfhydryl Groups in Relation to the Abscission of Cotyledon

by Yasuo Hotta and Takahisa Ôta[*]

堀田康雄・太田敬久： 生長素及び遊離 SH 基の子葉離脱における役わり

Under our standard germination conditions (27°C, room light ; Knop's sol.) the abscission of cotyledons of a bean, *Vigna sesquipedalis*, occurs on the 7th day of germination.

With a paper-chromatographic survey, it was confirmed that the substance positive to *Avena*-test in the cotyledon as well as in the hypocotyl is exclusively indole-acetic acid (IAA). In the epicotyl, however, abundant *Avena*-positive substan-ces other than IAA were detected in the early stage of germination, which were replaced later by IAA.

As to the content in diffusible auxins, a maximum was seen on the 3rd day in the epicotyl, and in the hypocotyl, although less conspicuous, cn the 1st or 2nd day. When the plants were grown in the dark, the content in the epicotyl showed a monotonous increase for 7 days without any peak, and, notwithstanding this, the time of abscission of the cotyledon was not changed. Diffusible IAA was absent in the cotyledon, and no IAA appeared to diffuse from the cotyledon into the stem. These negative results were likely not to be due to the breakdown of IAA by IAA-oxidase in the cotyledon tissues.

The ether-extractable IAA in the cotyledon and in the hypocotyl showed maxi-ma on the 1st day and on the 3rd, respectively, but that in the epicotyl showed a continuous increase. In the dark culture the extractable IAA content in these tissues showed a similar change to that in the room light culture. It was noted, however, that in the hypocotyl after the maximum was passed the content dropped

* Biological Institute, Faculty of Science, Nagoya University, Nagoya, Japan

much remarkably in the dark. In this case, also, no alteration was seen in the time of cotyledon-abscission (abscission on the 7th day).

It may thus be concluded that the abscission of the cotyledons would have no direct correlation with the content of auxins, either diffusible or extractable, in the stem as well as in the cotyledon tissues.

On the other hand, according to Muir and Hansch,[1] IAA is thought to be physiologically active when associated with the sulfhydryl (SH–) and amino groups of a specific protein. Van Overbeek et al.[2] have found that abscission of the foliage leaves is promoted by the administration of maleimides to the tissues in question. We have then treated the cotyledon with SH-blocking reagents, e. g., N-(1-naphthyl)-maleimide and monoiodoacetic acid, and have found that abscission of the cotyledon is accelerated by one and a half days. Noticeable was that the decrease in the extractable IAA content in the treated cotyledons was markedly slowed down. The content of free SH-groups in the cotyledon, as assayed spectrophotometrically by the method of Flesch and Kun[3] for tissue homogenates, decreased as the seedlings grew. The rate of decrease in the free SH-group content was also reduced by the SH-blocking reagents. Similar results were obtained with anti-auxins or by removal of the epicotyl and plumules. In these cases distinct dissolution of intercellular substances in the abscission layer, in spite of incomplete formation of the abscission layer itself, was found to take place.

Thus it is clear that the occurrence of cotyledon-abscission can also be not ruled by the SH-levels in the cotyledons and in the stem.

Further, no relation between the concentration gradient in respect to SH-group as well as IAA in the abscission region and the abscission time was seen.

We, therefore, wish to propose the following hypothesis as to the mechanism of cotyledon-abscission. Cotyledonous IAA is sucked in its bound form with SH-substance to the stem tissues. When this transport is hindered by some means, the "sucking factor" of the stem should be forced to be in an unsaturated condition, causing some critical changes in the metabolic pattern in the abscission region. These changes would induce abscission of the cotyledon.

In favor of this view, by means of histochemical techniques* it was found that more SH-groups in the parenchymatous cells were present in the cotyledons treated with SH-blocking reagents than in the normal tissues, and that the transport of substances in general from parenchyma into vascular bundles was blocked.

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* Investigated by the techniques of Bennet[4] and Barrnett and Seligman[5] with slight modifications to prevent and cancel contamination by metallic ions and thiophene.

花 粉 の 生 理 学 的 研 究

高 見 亙*

Wataru TAKAMI: Physiological Researches of Pollen

花粉の発芽に対する刺戟物質については多くの研究があるが, 無機塩の影響については徳川[8], Brink[1] によつて悪影響があることが示されて以来, 殆んど報告されてなく, ただ Loo & Hwang[5] は $MnSO_4$ が好影響を与えることを報告している, 筆者[7]は蓚酸カルシウムの結晶の晶癖に対してイオンの影響があることを知つたので, 花粉の発芽に対する 各種の イオンの影響について 観察し, 多くのイオンは適当な濃度では反つて好影響を与えることがわかつた。さらに, 花粉における澱粉粒や滲透圧に関して 得られた 結果を報告する。

実 験 の 結 果

(1) 花粉の発芽に対する無機塩の影響

(a) レンゲツツジ Control として蔗糖寒天

板 (蔗糖 10%, 寒天 1.5%, pH. 6.0〜6.2) にゝいて, 25〜27°c の暗所で発芽させると 3 時間位で発芽し始める。Fe, Sn, K, Mn, Mg, Ca, NH_4, Na, Zn, Co, Ba, Sr, Cu の塩化物 (Fe は $FeCl_3$ を使う) 及び $AgNO_3$ を 0.00001, 0.0001, 0.001, 0.01, モルにして 5 時間後に, ミクロメーターで 20 個以上の花粉管の 長さを 測つた。(11 目盛が 150μ.) Control は 4〜5〜7 目盛 (最小は 4, 中位数は 5, 最大は 7, 以下このように表わす) で, 測定の結果は表 1 のようであつた。

(b) エンドウとキショウブで前と同様にして試みるとエンドウでは 0.5 時間, キショウブでは 1 時間位で発芽し始めるから, 前者では 1 時間, 後者では 1.5 時間後に測定した。エンドウでは花粉管の先端で破裂するものが多く, 統計表はえにくいが, Control のは 4〜6 目盛 (11 目盛が 150μ) であるのに, 0.001, 0.0001 モル KCl の場合は 5〜7 目盛, 0.0001, 0.00001 モル $FeCl_3$ の場合に 5〜9 目盛と好影響がみられ, その他の塩は悪影響のようであつた。

キショウブでは花粉管の伸長速度は甚だ変動が多く, Control の場合にも 60〜210μ のようにミクロメーターで測定可能のものから 382〜693μ のように描画して測れたものまであり, $CuCl_2$, $AgNO_3$ 以外は主として 0.0001, 0.00001 モルの場合に好影響がみられ

表1. レンゲツツジの花粉管の長さ

(11が150μ, Control は4〜5〜7.(最小が4, 中位数が5, 最大は7, 表中もこの表わし方による))

		0.00001モル	0.0001モル	0.001モル	0.01モル
(1)	$FeCl_3$	6 ~ 8 ~10		20~25~32	不 発 芽
(2)	$SnCl_2$	22~24~30	10~13~20	5~7~10	不 発 芽
(3)	KCl	18~22~30		10 ~ 12 ~ 16	不 発 芽
(4)	$MnCl_2$	7 ~ 9 ~13			2~3~4
(5)	$MgCl_2$	3 ~ 4	8~10~15	6~8~11	1~3
(6)	$CaCl_2$	4 ~ 6 ~ 7		15~17~30	8~10~18
(7)	NH_4Cl	10~12~20	5~7~10	4 ~ 6 ~ 7	
(8)	NaCl	5~7~9	7~9~15	5~7~9	不発芽多い
(9)	$ZnCl_2$	6~8~11	1 ~ 4		不 発 芽
(10)	$AgNO_3$	4~5~7	1~3~6	不発芽	不 発 芽
(11)	$CoCl_2$	4~6~8	2~4~5	不発芽	不 発 芽 と 破 裂
(12)	$BaCl_2$	3 ~ 5	2 ~ 5	2 ~ 4	不 発 芽
(13)	$SrCl_2$	不発芽	不発芽	不発芽	不 発 芽
(14)	$CuCl_2$	不発芽	不発芽	不発芽	不 発 芽

* 早稲田大学商学部生物学教室 (Biological Institute, Faculty of Commerce, Waseda University, Tokyo)

た。（$CuCl_2$ も 0.00001 モルの場合には好影響を与える）。例えば，$Fe Cl_3$ では 2411, 3882μ，$CaCl^2$ では1764, 1882μ，$Zn Cl_2$ では 1088, 2088μ，KCl では 852, 1177μ，$MgCl_2$ では 705, 1441μ，$Cu Cl_2$ では 835, 1205μ のようである。KCl, $CuCl_2$, NH_4Cl, $SrCl_2$, $MgCl_2$ では 0.01 モルでも好影響がみられた。また，当然推定されるように原形質流動速度も影響をうける。花粉管の長さが350μ のもので Control の場合の速さは 1.1μ/sec. 0.0001 モル $FeCl_3$ の場合の速さは 1.4μ/sec. であつた。

（c）**ビョウヤナギ** これは水でも発芽する場合があるから寒天が薄い方が良好で，Controlとして蔗糖寒天板（蔗糖10%，寒天 1%，pH. 6.0〜6.2）にまいて，室温 23°C の明所で発芽させると 2 時間位で発芽し始める。不発芽または破裂の悪

影響がみられ，その程度が少ないものは $SnCl_2$, $CoCl_2$, $MnCl_2$ で，程度の大きいものは $CaCl_2$, $CuCl_2$, $ZnCl_2$ $BaCl_2$, $AgNO_3$ であり，その他は，Control で 2〜7〜15 目盛（13目盛が 150μ）であるのに対し表2のような結果がえられた。

また，ホールグラスによつて Control として10%蔗糖液（pH. 5.6）を使い，懸滴の方法で0.01 モルの塩によつて調べると 26°C において，Control は 2〜5 目盛（13目盛が 150μ）で発芽率は少ないのに，$Fe Cl_3$ では pH 4.6 の場合は発芽率 100%で，pH 5.6 の場合にも甚だよく，最大の長さは37目盛にも及んだ。好影響があると認められたのは KCl（最大11目盛），NH_4Cl（最大16目盛），$CuCl_2$（最大15目盛），$Zn Cl_2$（最大17目盛）であつたが $Fe Cl_3$ の場合を除いて発芽率が少なく，寒天板における程はつきりとはしない。

（d）**ホウセンカ** 水のみで発芽するので，ホール・スライドに水（pH 5.2）と 0.000001, 0.00001, 0.0001 モルの$FeCl_3$を滴下し，25°〜26°C で，同じ葯の花粉をまき，20分後に 20 個ずつの平均を求めると表3のようであつた。

（e）**チヤ** 水のみで発芽するので，ホール・スライドに水（pH 5.2）と 0.000001, 0.00001, 0.0001モルの $FeCl_3$ を滴下し，19°C のとき，同じ葯の花粉をまいて 1.5 時間後に 20 個ずつの平均を求めると表 4 のようであつた。

（2）蔗糖の発芽に対する影響

ビョウヤナギは場合によつては水だけで発芽するから水及び10%の蔗糖を使つて懸滴の方法で同時に発芽させると1.5時間後では，両者とも 11〜21目盛（13目盛が 150μ）で変らないが，15時間後には 10 個について，

表 2.　ビョウヤナギの花粉管の長さ
（13が 150μ, Control は 2〜7〜15）

		0.00001モル	0.0001モル	0.001モル	0.01モル
(1)	$FeCl_3$	7 〜 12 〜 18		24〜40〜44	13〜35〜44
(2)	KCl	3〜5〜8	5〜8〜18		4〜15〜22
(3)	NaCl	11〜18〜28	11〜15〜20	6 〜 11 〜 18	
(4)	NH_4Cl	8 〜 17 〜 20		5 〜 8 〜 11	
(5)	$MgCl_2$	5 〜 10 〜 12		11〜 16 〜 24	

表 3.　ホウセンカの花粉管の長さ（20 個の平均，
13 が 150μ, 第1〜3回が25°C, 第4〜6回が26°C）

回	水	0.000001モル	0.00001モル	0.0001モル
1	11.3	15.3	20.8	破裂
2	2.7	12.0	9.9	9.2
3	3.3	11.1	12.0	8.5
4	4.7	6.3	6.4	殆ど破裂
5	2.5	5.1	8.7	5.8
6	5.0	7.3	11.1	11.8

表 4.　チヤの花粉管の長さ（20 個の平均 13 が 150μ.）

回	水	0.000001モル	0.00001モル	0.0001モル
1	3.9	7.1	5.8	4.3
2	7.2	9.7	11.7	7.3
3	7.1	15.9	8.1	4.2
4	6.6	9.5	10.2	4.5
5	7.4	9.1	8.9	6.6

水の場合には最小 10,最大40,平均 21.1であるのに，蔗糖の場合は最小72,最大100,平均81.5 で花粉管の伸長は著しく異なる。そして，花粉粒の形は Fig. 1. のように，水の場合には縮んでしまう。

Fig. 1　ビョウヤナギの花粉の水と10% Sucrose による発芽，15時間後の状態，左方が水，右方が Sucrose　×200

（3）　滲透圧と澱粉粒

（a）　ムラサキツユクサ　この花粉では負の滲透圧現象がみられるものもあり，長径が70μ位のものの滲透圧は蔗糖の約0.1モルで，115μ 位のものでは約 0.2モル，150μ 位のものでは約 0.3 モルとなつてしだいに滲透圧が増し，若いものは空胞が大きく，成長後に小さくなる。(Fig. 2.)

次に，花粉内の澱粉粒は花粉が成長する場合に消費されて急に減少することが観察される。Fig. 3. は長径 75μ，87μ のものおよび 116μ，150μ のものの澱粉粒を比べたものである。

（b）　エンドウ　5月8日頃まで蔗糖 10％の寒天板でよく発芽したが 5 月 11日には蔗糖 16〜18％でないと破裂した。

（c）　キショウブ　雨天の日の花粉は蔗糖を含まない寒天板でも少数発芽したが，晴天の日のものは全部破裂した。

（d）　ビョウヤナギ　：雨の日に枝をとつて翌日咲いたものは10％の蔗糖水で懸滴の方法でよく発芽したが，晴れた日にとつて翌日咲いたものは12％の蔗糖水で大部分破裂した。(Fig. 4)

（e）　ツキミソウ　この場合にも同様な傾向が見られた。

（4）．帽体

ビョウヤナギの貯蔵物は脂肪で蔗糖寒天板で発芽させると，澱粉粒は帽体近くまで殆ど一様に分布され，帽体の所は管が短いものは原形質が凹形をなしているものが多く，十分伸長した後は凸形になり，甚だ長く伸長した後では帽体に澱粉粒が見られない。また，ツキミソウで管に突起が出来たものは帽体の所で成長して行くこと[2]が観察される。この場合に澱粉粒は原形質流動によつて帽体に入るが消失して戻つてこないものがある。ノカンゾウは蔗糖寒天板では伸長が不連続的で，先端が次々に附加されて行き，縞のようになる。(Fig. 5.)

Fig. 2.　ムラサキツユクサの花粉 a. 負の滲透圧現象 b, 長径70μ のものの 0.1モル Sucrose での原形質分離，c. 同じく長径 115μ，0.2 モルの場合，d. 長径 150μ，0.3モルの場合　×150

Fig. 3.　ムラサキツユクサの花粉内の澱粉粒，長径は左から 77μ，85μ，116μ，150μ　×150
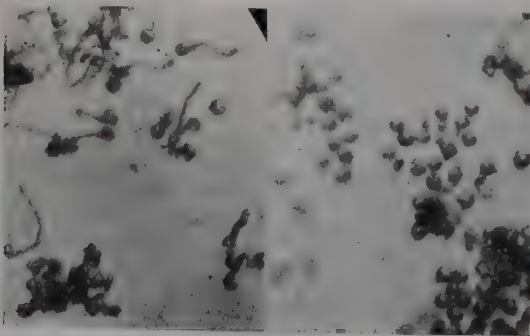
Fig.4.　ビョウヤナギの花粉の滲透圧の変化 a. 12％の蔗糖水で大部分破裂，b. 10％の蔗糖水で発芽　×110

結論及び論議

上の実験の結果によると
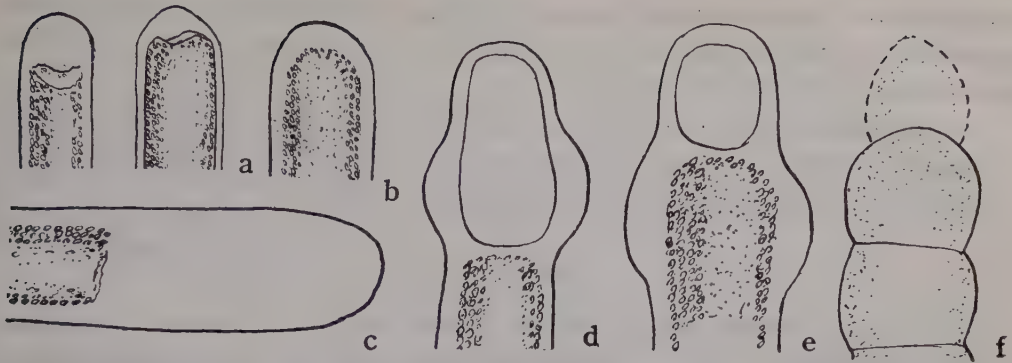
Fig. 5. 帽体の形状，a. ビョウヤナギの短い花粉管の凹形のもの　b. 長い花粉管の凸形のもの，c. 十分長いもの，d, e. ツキミソウの花粉管の伸長の相続いた二つの形状，f. ノクワンゾウにおける先端附加（点線の部分）a. b. c. ×1500, d. e. f. × 320

多くの無機塩は適当な状態においては花粉管伸長の刺戟物質となることが知られる。 5％の蔗糖水に0.001％の無機塩を添加した場合に悪影響が見られたのは[8]，未だ状態がよくなく，塩の影響が強すぎたためであろう。この実験でも蔗糖寒天板の場合の方がはつきりした影響がみられた。2.4-Dの場合に濃度が小さい場合に促進作用が報告されているが[3]，同様な意味をもつていると考えられる。ツツジの場合には $FeCl_3$, $SnCl_2$, KCl, $CaCl_2$, NH_4Cl が好影響を与え，$MnCl_2$ も多少好影響である。キショウブではさらに $NaCl$, $SrCl_2$ なども好影響を与えたが，この場合には伸長が敏感で変動があり，ある場合には 1.5％の寒天板に炭酸水 10％程添加すると Control の $300\sim600\mu$ に対し 2000μ 位になつたことがある。ビョウヤナギでも蔗糖水10％だけで懸滴で試みると発芽率が50％以下であるのに炭酸水少量を加えると 100％になつた。チャやホウセンカの場合には水に微量の

$FeCl_3$ を添加すると花粉管の伸長が促進されることが認められた。

次に，陰イオンによつて差異かあるかを調べるのに，キショウブでは KCl, KNO_3, K_2SO_4 においては著しい差は認められなかつたが，ビョウヤナギでは KNO_3, K_2SO_4 は KCl に比べて害作用が強いようである。

花粉の発芽に対する糖類の作用についてはすでに Brink[1], 岩波[4], O' Kelley[6] らによつて述べられているが，ビョウヤナギのような小形の花粉では周囲からの養分が必要であることがはつきり認められた。また，花粉の発育に際して滲透圧は急に大きくなり，生長した花粉も天候に応じて滲透圧を自己調節している[4] と考えられる。澱粉粒の分解反応によつて花粉粒の大きさの増大や，花粉管の伸長が行われることはすでに岩波[4] によつて報告されてはいるが，二三の観察の結果を附加した。

Summary

In the present investigation, in the first place, the effects of various inorganic salts on pollen tube growth were observed.

Used salts were $FeCl_3$, $SnCl_2$, KCl, $MnCl_2$, $MgCl_2$, $CaCl_2$, NH_4Cl, $NaCl$, $ZnCl_2$, $CoCl_2$, $BaCl_2$, $SrCl_2$, aad $AgNO_3$. Observed flowers were *Rhododendron japonicum*, *Ptsum Sativum. Iris Pseudacorus, Hypercium chinese Impatiens balsamina* and *Thea sinensis*,

Conirary to commonly believed results, on suitable conditions, growth-promoting effects on pollen tube of some salts, such as $FeCl_3$, $SnCl_2$, KCl, $CaCl_2$, NH_4Cl etc.

were observed.　In case of agar media containing 10 percent cane sugar, optimum concentration may be 0.00001–0.0001 mol, but in case of water media it should be more dilute.

In the second place, observations about function of sugar, about relation between starch grains, osmotic pressure and pollen tube growth, and about daily change of osmotic pressure of pollen grains were done.

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シダの胞子の発芽に及ぼす光の影響　II
オシダの2回の光期について

石 川 茂 雄*　　　大 房 　剛**

Sigeo IsIKAWA and Tuyosi OOHUSA: Effects of Light upon the Germination of Spores of Ferns II.　Two Light-periods of *Dryopteris crassirhizoma* Nakai

シダ類に関する研究は前葉体について為されたものが多く，発芽，特に光の影響についての報告は極めて少い。

最近では Steevés 等のワラビ (*Pteridium aquilinum*) を用いた畸型の前葉体形成[10]及びその際の核分裂についての報告があるが，これも胞子の発芽にはふれていない。又，Castle はスギナ (*Equisetum arvens*) で前葉体の生育を観察した報告の中で，胞子の発芽が炭素源として砂糖を，窒素源として casein hydrolysate を添加した時に早められる事を述べている[1]。しかし，この実験は全光下で行つており光の影響は考えていない。ただ岡田によつてシダの胞子も発芽に光が必要である事が報告されている[7]。

著者等は先に毎日の照射時間と発芽率の関係を報告し，シダの胞子が発芽する際にも種子の発芽におけると同様に「光週性」的な現象が認められ，その傾向は大部分の種子がいわゆる「短日性」であるのに対し，シダにおいてはほとんどが「長日性」を示し，少数の種類でのみ「短日性」が認められた事を述べた[4]。今回は典型的な「長日性」を示すオシダ (*Dryopteris crassirhizoma*) を選び，その2回の光期について報告する。

実験材料及び方法

胞子は 1955年9月16日に山梨県入笠山で採集した。オシダはシダ類の胞子中最も寿命が長いものの一つで，約 6ケ月にわたり初期の光感性がたも

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　* 東京教育大学理学部植物学教室　Botanical Institute Fac. of Sci., Tokyo Univ. of Education
** 東京都立大学理学部生物学教室　Department of Biology, Fac. of Sci., Tokyo Metropolitan Univ.

れたが，此の実験はすべて採集後 1～3
ケ月以内に行つた。

　実験方法は 4.5cm シャーレに 1 ％寒
天液を注ぎ，固つた後に胞子をその面上
に細筆でうすくまき，直ちに黒いラシャ
紙で個々のシャーレを包み，18±1°にた
もつた。最後の光照射が終つてから 7 日
目に顕微鏡で 150 倍の 1 視野中に見られ

Day-length　in hrs.
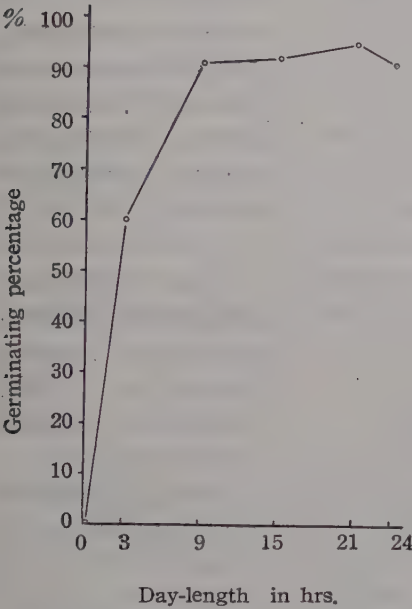
Fig. 1　Daily exposure to light
of different duration.

Duration of dark period (D₁) in hours.

Fig. 2　Effects of the length of dark period
(D₁) inserted two times of 6 hours'
illumination at different presoaking
time (Dp).

Presoaking time (Dp) in days

Fig. 3　Change of degrees of light-sensitivity with
lapse of presoaking time. one light period;
18 or 24 hrs.

た発芽数と全胞子数をかぞ
え，5 視野の平均をとり発
芽率とした。

　光源には正午の太陽光線
とほとんど同じ特性曲線を
持つマツダ天然昼光色螢光
燈を用い，シャーレの上面
で 600 Lux とした。

　　　実 験 結 果

I.　光照射の時期と間隔

　オシダは第 1図に示す如
く，毎日一定時間の光照射
を 7 日間繰返すとその照射
時間が 9 時間であれば最高
の発芽率に達し，全光下で

も発芽率が低下しないいわゆる「長日性」である。
しかし，この様な発芽率を得るためには 7 回もの
繰返し照射が必要ではなく，その間におかれた暗
期 (D_1) の長さが 18 時間であれば 2 回の光照射
(L_1, L_2) のみでよい。この D_1 の長さはまいてから
L_1 を照射し始める迄の暗期の長さ (D_p) には関係

しないが，D_p の長さにより発芽率が異る。これ
は胞子の光感性が D_p の時間によつて変るために
起ると考え，18 時間又は 24 時間 1 回の光照射を
行つて光照感性の変化をみた（第 3 図）。

以上から，以後の実験は光感性が高く，しかも
安定している播下後 24 時間目から行う事とした。

第 4 図に，L_1, L_2 の長さ
をそれぞれ 3 時間，6 時間，
9 時間とし，各々について
D_1 を 0 から 30 時間迄延し
た際の発芽率の変化をしめ
した。即ち，L_1, L_2 の長さ
に関係なく最も高い発芽率
を示す D_1 の長さは 12～18
時間である。

II. L_1, L_2 の長さ

以上から D_1 の長さは 15
時間前後が最適である事が
認められたが，この長さの
暗期をはさんだ L_1, L_2 の長
さを種々に変え，各種の組
合せを作つた。

まず，L_1 と L_2 の和を 6 時
間とした場合（第 1 表）は，
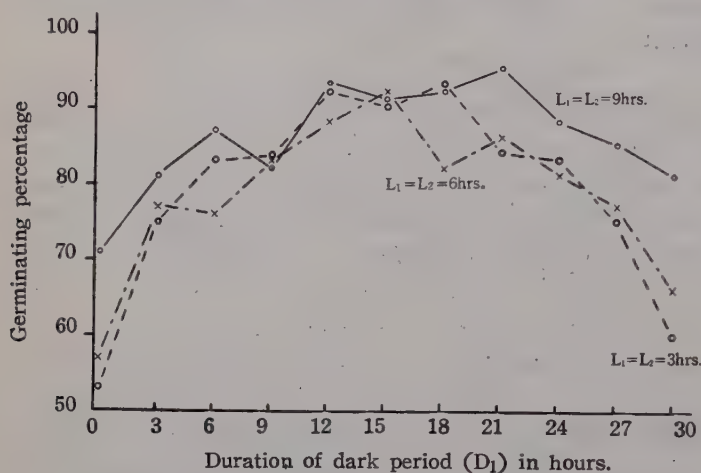
Fig. 4 　Effects of the length of dark period (D_1)
inserted two illuminations.

　　——　　　$L_1 = L_2 = 9$hrs.
　　— · —　$L_1 = L_2 = 6$hrs.
　　— — —　$L_1 = L_2 = 3$hrs.
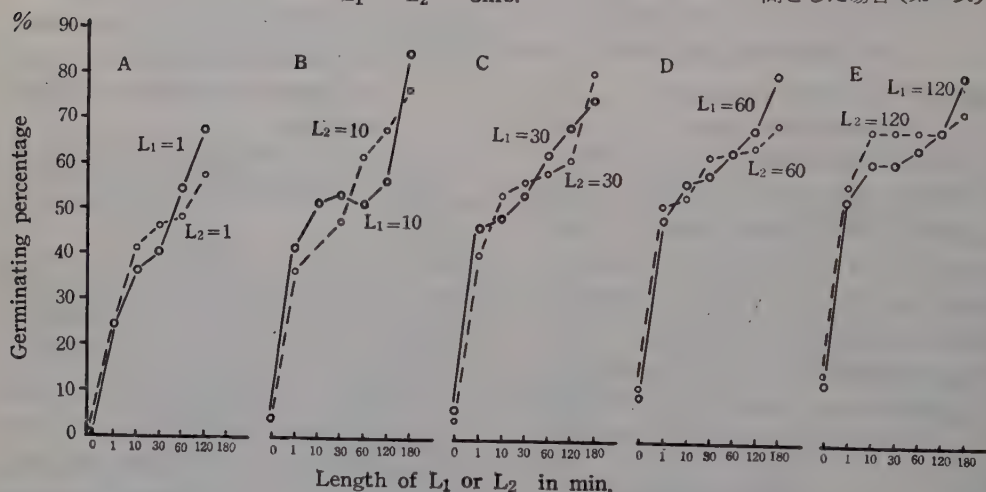
Fig. 5 　Effects of two light periods of different duration.
　　——　 First illumination was uniformed, and exposed
　　various length of illuminating time into the second
　　light period.
　　— — —　 Second illumination was uniformed and exposed
　　various length of illuminating time into the first
　　light period. (in min.).

6 時間唯 1 回の照射では 53％にしか達じたい発芽率が同一照射時間を L_1, L_2 の 2 回にわける事により, L_1, L_2 の長さにかかわらず最高の発芽率に達した。これは 2 回の光期と暗期 (15 時間) との和である 21 時間連続照射した際の発芽率より高い。

さらに第 5 図は L_1 を 1〜120 分とし, その各々について L_2 を 1〜120 分照射した結果である。実線は L_1 を一定として L_1 を種々に変えたものであり, 点線は色々の長さの L_1 を与えたのちに一定時間の L_2 を照射したものである。例を L_1 及び L_2 が 1 分間の場合 (第5図A) にとれば, L_1 を 1 分間照射したのちに L_2 を 0〜120 分照射すると, 発芽率は L_2 の増加につれて 0, 24, 36, 40, 53, 67％と増す。逆に 0〜120 分の L_1 を照射したのちに 1 分間の L_2 を与えるとその発芽率は 0, 24, 41, 46, 48, 59％となり, 2 つの線は一致し, 発芽率が個々の光期の長さに関係せずに 2 回の光期の和によつて決められていることを示す。

考　察

オシダは第 1 図に示めされる様に典型的な「長日性」に属するものであるが, 唯 1 回の光照射を行つてもその時間さえ長くなれば高い発芽率に達する (第 3 図)。この結果得られた光感性の波状変化はタバコの種子によつても示された[3] ものである。しかし, タバコでは高い光感性が長い Dp の後にも短時間唯 1 回の光照射で得られたのに対し, オシダでは播下直後から高い光感性が示めされ, Dp を長くすれば直ちに低下してしまう。しかも, かかる光感性を得るためには24時間という長い光照射を必要とする。この長い光期はイワアカバナ[5] (Epilobium cephalostigma) と同様 2 回の短い光期にわける事が出来, D_1 の最適時間は L_1, L_2 の長さに関係なく 12〜18時間である (第4図)。いま L_1 及び L_2 を 3 時間とした時の変化をみると, D_1 が 12〜18 時間の時の発芽率はいずれも 90％に達しているが, 21 時間になると64％に低る。これが毎日 3 時間づつ 7 日間繰返し照射 (この時の暗期は 21 時間) を要つた際の発芽率 60％ (第1図) と一致することは非常に興味深い。

さらに, 第 4 図から発芽率は L_1, L_2 を 3 時間として D_1 を 6 時間とした時 (光期と暗期の和は 12 時間) すでに 83％, D_1 を 12 時間とすれば (総和

は18時間) 90％に達し, 12時間光を照射し続けた時に57％, 18時間の連続照射が 71％であるのに較べ, はるかに高い。これは, 此の胞子が全光下でも発芽率が低下しない「長日性」であるにも関らず, 暗期の挿入によりかえつて発芽の機構が促進されていることを示している。

又, 第 1 表及び第 5 図に示す様に発芽率は L_1, L_2 の各々の長さには関係せず, 2 回の光期の和 ($L_1 + L_2$) で決められる。第 5 図から L_1, L_2 の和が大体 60 分の場合をえらぶと (第 2 表), いずれの組合せでもその発芽率は 60 分ただ 1 回照射の際 (10％) よりはるかに高く, 大体 50％前後を示す。しかも, この発芽率は L_2 又は L_1 が 1 分間の時にも同様である。

かかる発芽率が L_1 と L_1 との和によつて決められると云う現象は,「短日性」のイワアカバナで L_1 が短時間の照射で充分となり, L_2 の長さにより発芽率が決められる[5] 事と非常な相違を示す。

又, 以上述べてきた様な 2 回の光期の結果は, 小河原[6] によるタバコの報告と外見上は一致する所が多い。しかし, すでに Raciborski[9] (1900) の報告以来タバコの種子は極めて短時間の光照射で発芽する種子の代表とされて, Crocker[2] もタバコの様に発芽が極めて少量の光量でたりるものでの光の機構が他の多量の光量を要するものと異つているのであろうと述べている。さらに石川も[3], 小河原と同じ黄色種を用い 1/90 秒ただ 1 回 150 Lux の光を照射したのみで 54％の発芽率をみており, タバコの種子中では比較的光感性が低い国分種でさえも 1000 Lux 1 分間の照射で 64.8％の発芽率を報告している。この様にタバコはただ 1 回短時間の光照射を行つたのみで発芽の機構が完了するものであり, 小河原の報告は根本的な発芽機構の上からも賛成し難いものがある。

結　論

典型的な「長日性」であるオシダはただ 1 回の光照射を行つてもその時間が18〜24時間になれば高い発芽率に達する (第 3 図)。此の 1 回照射によつて得られた光感性の変移は波状の変化を示す。

かかる 1 回の長い光期は 2 回の短い光期にわける事が出来る。この間に入れた暗期の長さは 12〜18 時間が最適であり, この時間 (D_1) は光期の長さに関係しない (第4図)。又暗期の存在はその時期にも続けて光照射を行つた際より高い発芽率を

示し，暗期による発芽機構の促進が認められた。

　さらに，発芽率は光期個々の長さには関係せず，2 回の光期（L_1+L_2）の和で決められる（第 1 表，第 2 表，第 5 図）。

　以上のような種々の傾向は同じ「長日性」を示すシケシダ，ホシダにも見られるので，此等が

「長日性」を示すシダの胞子の 一般的な 性質であると考えられる。

Table 1.　Effects of two light periods of different duration at optimum dark period of 15 hours.

Length of light	L_1	6	5	4	3	2	1
period (hrs.)	L_2	0	1	2	3	4	5
Germinating percentage	%	53	90	85	90	86	91

Single illumination 21 hours: 85 %

Table II.　Effects of two light periods of different duration at optimum dark period of 15 hours.

Lenght of light	L_1	60	60	60	30	10	1	0
period (min.)	L_2	0	1	10	30	60	60	60
Germinting percentage %		10	48	56	53	53	51	11

Résumé

　Experiments were performed to make clear the effect of light and darkness on germination of the spores of *Dryopteris crassirhizoma* which have a typical character of "Long Day Seeds".

　1)　A high germination rate was obtained by a single illumination so long as its duration is fairly long, for exmple, 18 or 24 hours. However, the illumination on the 4th day in the presoking time had rather weak effect on the germination, as the light-sensitivity changed with the lapse of presoaking time, as observed in the case of tabacco seeds (Fig. 3).

　2)　One long light period mentioned above was divided into two short light periods, L_1 and L_2 with insertion of one dark period D_1. With varying dark periods, it was revealed that the dark period optimum for germination was 12–18 hours, regardless of the lengths of both light periods.

　3)　The germination rates secured by insertion of a dark period were higher than the rates obtained with one continuous exposure to light whose length was

equal to the total period of $L_1+D_1+L_2$. Therefore, it might be concluded that the dark period inserted between two light periods promoted the germination of the spores positively.

4) With various combination of L_1 and L_2 and an optimum dark period of 15 hours, it was recognized that the germination rates were generally increased in proportion to the total length of both the light periods, and had no direct relation to each length of them. In this photo-germinating character, the spore of *Dryopteris crassirhizoma* is obviously different from the seed of *Epilobium cephalostigma*.

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ツルナシインゲンの幼胚におけるコハク酸脱水素酵素とミトコンドリャの分布について[*]

佐　藤　七　郎[**]

Sitiro Sato: The Distribution of Succinic Dehydrogenase and Mitochondria in the Embryos of *Phaseolus vulgaris*.

1. まえがき

著者は前報において，ツルナシインゲンの未発芽種子の幼軸および幼根におけるコハク酸脱水素酵素活性の組織化学的検出の条件についてくわしく検討を加え，その結果にもとずき，この酵素の活性が分裂組織につよく，なかでももつとも未分化な前形成層の細胞に，他に先んじて検出される

ことを明かにした。従来，高等植物の組織におけるこの酵素の分布については，くわしく検討をくわえたものがない。わずかに形成層をもふくめた分裂組織がメチレン青，TTC (2, 3, 5 -triphenyl tetrazolium chloride) を還元する傾向がつよいことが知られているにすぎない[3, 9]。著者の実験でも，TTC が直接にどんな酵素で還元されるか

を結論するまでには至つていないが, すくなくも コハク酸脱水素酵素が関与している系が TTC を 還元し, それが前形成層に分布していることは結 論できるとおもわれる。

著者の実験条件におけるコハク酸脱水素酵素反 応分布の一例を Fig. 1 にしめす。これを幼軸, 幼根にわたつて模式的に図示すれば Fig. 2 左半 のようになる。
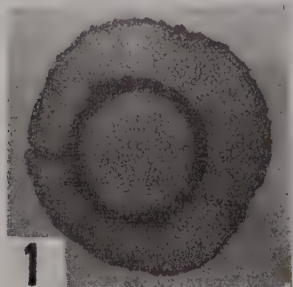
Fig. 1. Transection of the hypocotyl region. Procambium shows the strongest reduction of TTC.
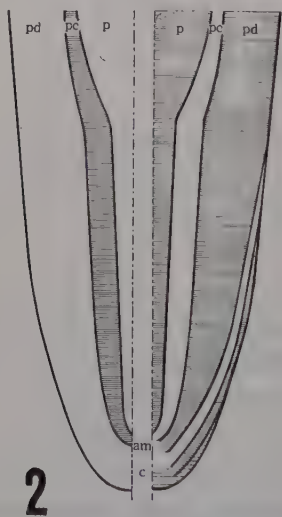
Fig. 2. Diagram of a longisection showing the reverse distribution of succinic dehydrogenase reaction (left) and mitochondria (right) (shaded). The enzyme reaction is strongest in procambium, while the mitochondria are distributed in pith (prelome except procambium) and periderm. p, pith; pc, procambium; pd, periderm; am, apical meristem; c, calyptrogen.

他方, 近年の研究によれば, コハク酸脱水素酵 素は連関する諸脱水素酵素およびチトクローム系 とともに, 細胞質内の大型顆粒, なかでもミトコン ドリヤに結合している事実が明かにされ, 高等植 物においてもしだいに一般化されるにいたつた, もしこのことが著者の実験材料においても適用さ れるならば, ミトコンドリヤの分布は TTC 反応 部位と一致するはずである。著者はこの点を確認 するために, 幼胚におけるミトコンドリヤの分布 を固定像およびヤヌス緑 B による生体染色によつ てしらべた。

2. ミトコンドリヤ固定像の分布

観察方法　浸水ご約20ジカンの胚から子葉をは ずし, 下胚軸と幼根を Champy 固定液で24ジカ ン固定。水洗ごアルコール, クロロホルムをへて 65°C でパラフィンを誘導。10μ の厚さに輪切り切 片をつくる。スライドグラスに貼布, 風乾ご, キ シロールでパラフィンをのぞき, アルコールをと おし, 鉄ミョーバンを媒染剤としてヘマトキシリ ンでそめる。

おそらく固定液とクロロホルムの中に入つてい る時間がながかつたため, 試料はたいへん固くな つた。そのために切片は部分的に組織がこわれて いる。しかしこれはミトコンドリヤの分布をたし かめるのにさしつかえるほどではない。(そのご ミトコンドリヤがうまく固定されないばあいがよ くあつたので, 固定のためのよい条件について検 討を加えているが, 固定を過度に行うことがひと つの必要条件であるらしい。)

結果: ミトコンドリヤは立体的に曲りくねつた 短い糸状の像として青くそまつて現れる(Fig. 3)。
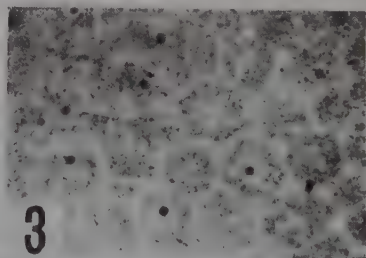
Fig. 3. Mitochondria in peridermal cells. Fixation, Champy. Stain, iron hematoxylin.

曲つているので長さはわかりにくいが種々の長さ のものがあるようにみえる。太さは同一の組織の 中の細胞ではほぼ一様である。

この像はサク酸，アルコールなどの有機溶媒をふくんだ Carnoy, Bouin, Nawaschin などの固定液ではあらわれない。また Champy の固定液を用いても現れないばあいがある。好適な固定のためにはふくざつな条件が必要のようである。これはミトコンドリヤのきわめて不安定な性質と関係があろう。

1 個の細胞内においては細胞質中に均等に分布し，核の中にはかかる像はみとめられない。核の中には仁だけがそまっている。

これらの染色性は，この像が細胞学において形態学的に定義されたミトコンドリヤであることを有力に示すものである。

このミトコンドリヤを含んだ細胞の組織内における分布は Fig. 2 右半のごとくである。すなわち原皮層およびズイの細胞内にもつとも明かに現れ，前形成層と根端分裂組織（原始細胞群）にはみられない。原皮層に接する前形成層の細胞，原表皮および原根冠の細胞にははつきりとしたミト

コンドリヤ像はみとめられないが，しかし前形成層のばあいとことなつて，微細な粒子がそまつている。この粒子は外見的には中間的な像であるが，これを度外視すれば，ミトコンドリヤを有する細胞と有しない細胞との対照はきわめて明瞭である（Fig. 4-8）。また例外はなく，原皮層およびズイの細胞はすべてこれを含み，前形成層と根端分裂組織の細胞はすべてこれを含まない。

このミトコンドリヤの分布は，予想に反してまえにのべた TTC 反応のつよい細胞の分布と正反対である。

3. ヤヌス緑Bによる生体染色

細胞内のある構造がミトコンドリヤであるかどうかを同定するためには，単一の方法では充分とはいえない。とくに固定染色像のばあい，人為構造が現れたり色素体や後形質と誤認する危険を排除するために別の方法，とくにヤヌス緑B(JG-B)による生体染色をたしかめる必要がある。JG-Bによる特異的生体染色は，古くからしられてもつとも広く用いられているミトコンドリヤ同定の方法である。なお，色素体や油滴も JG-B を吸着するといわれているが，sorokin[8] は，ミトコンドリヤはこれを無気条件下で還元脱色するが，色素体や油滴はそのような力をもたないので明確に識別されるといつている。

実験方法と結果：同じ材料を，TTC 反応を行わせるときと同様に切片とし，2～3枚ずつホールスライドで JG-B 水溶液(1：10,000) に浸す。このときカバーグラスをかけてもかけなくても，ただちに切片の周囲がミズ色に変り，やがて切片がミズ色にそまつてくる。1：100,000 ではほとんどそまらない。
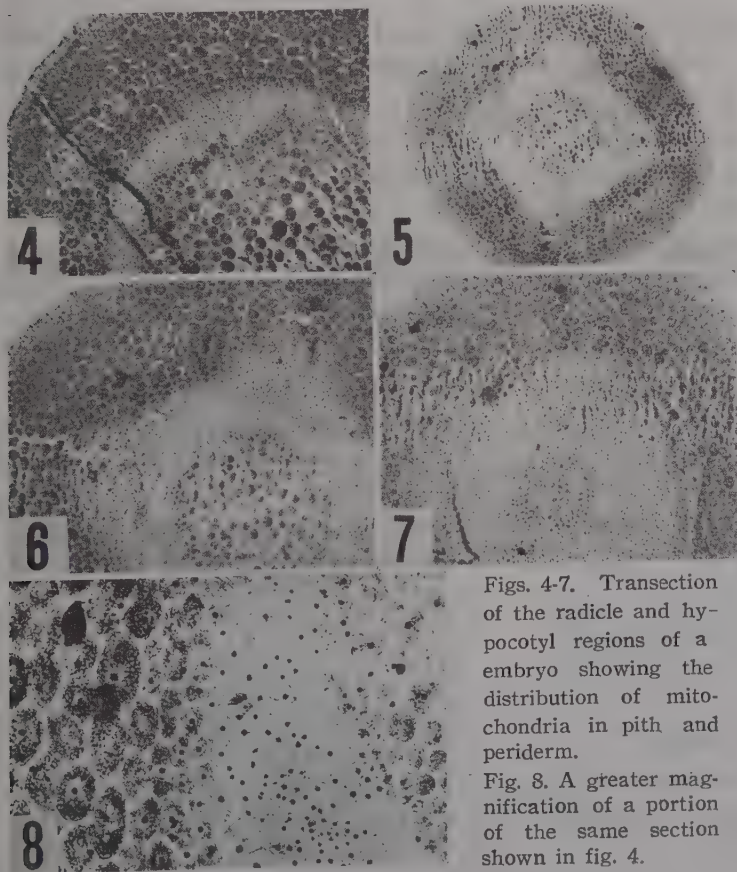
Figs. 4-7. Transection of the radicle and hypocotyl regions of a embryo showing the distribution of mitochondria in pith and periderm.

Fig. 8. A greater magnification of a portion of the same section shown in fig. 4.

染色部位は固定像の分布と一致し，原皮層とズイであつて，前形成層と根端分裂組織は白色半透明のままのこる。ただ表皮がつよくそまるのは唯一の不一致点である。

カバーグラスをかけないで放置すると，着色はますます強化し，長時間ごにはこいアイ色にそまる。しかしカバーグラスをかけ，とくにその周縁をワラツプなどで封ずると，まもなく変色がはじまり，前形成層以外のぶぶんがムラサキ色になり，ついでボタン色に変つてくる。長時間ごには完全に脱色して無色になる。このことは切片の JG-B 染色が色素体や油滴の染色によるのではなくミトコンドリヤによるものであることをしめす。なおこの変色，脱色の性質についての定性的研究の結果はすでに報告した[6]。

そまつた細胞を顕微鏡で観察したが，細胞が小型で観察しにくく，はたして細胞内のどの構造がそまつているのかを識別することは困難であつた。ただ核と細胞膜がそまつていないことだけがたしかめられた。

4. 細胞外にながれてたミトコンドリヤらしい顆粒の JG-B 染色

生の切片を水でしたてたプレパラートを暗視野で観察すると，外液に無数の微細な粒子が浮游して，さかんなブラウン運動をおこなつているのがみとめられる。この粒子は明視野ではみえない。しかしこれに JG-B 水溶液を注入してやると，粒子はこいアオ色にそまつて明視野でもよくみえるようになる。この粒子は，大さからいつても，また染色性からいつてもおそらく，切断された細胞から流れだしたミトコンドリヤであろう。このことからも，生細胞にそうとう多数のミトコンドリヤがふくまれていることが推定される。

5. 偏光顕微鏡所見

若い胚の幼胚軸などの原皮層とズイにデンプンの細粒がふくまれていることがある。これはヨード反応および複屈折によつて識別されるが，上記のミトコンドリヤは，固定像も生体像も複屈折を示さなかつたから，デンプンの誤認でないことは明かである。

6. 発芽後の胚軸の JG-B 染色

以上は発芽前の幼胚における知見であるが，発芽後の JG-B 染色の位置は逆転して，もつぱら形成層がよくそまる。固定染色像も分裂細胞によくみられることは，たいていの文献に報ぜられているから，形成層にミトコンドリヤがふくまれていることはまちがいないものとおもわれる。皮層，ズイなどはそまらないようにみえるが，これはミトコンドリヤがふくまれていないということではない。細胞が空胞化していて細胞質がすくないために，識別できるほどに色素を蓄積しないのだろう。近年，胚軸から生化学的にミトコンドリヤと同定される顆粒が比較的大量に単離されている事実[2,7,10]を胚軸が大部分は皮層とズイの細胞からなつていることと合せかんがえても，これらの細胞にミトコンドリヤがふくまれていることは否定できない。

7. 考察と論結

ミトコンドリヤはすべての細胞にふくまれているとか，あるいは若い未分化な分裂組織などにとくによくみられるなどといわれることが多い[1]。しかしこの観察では，発生の比較的初期の段階にある胚では，このことはあてはまらないことがわかつた。すなわち，この胚では，もつとも未分化の段階にある根端分裂組織と前形成層の細胞にはまだミトコンドリヤがみられず，かかる細胞が分化してできたズイ，原皮層，あるいは形成層，皮層の細胞になるとミトコンドリヤが現れるのである。

他方，コハク酸脱水素酵素の反応は，もつとも未分化の前形成層につよく検出される。この前形成層の細胞はまだミトコンドリヤをもつていないのであるから，コハク酸脱水素酵素は，すくなくも可視的な構造には結合しない状態で存在するものとかんがえられる。細胞の分化がすすむにつれじ可視的なミトコンドリヤ像が現れてくるにしたがい，この酵素はミトコンドリヤにふくまれるようになることが想像される。ただしこの過程については途中の推移を追跡することによつて，さらに検討を加えなければならない。

Summary

In the earliest stage of germination (after about 20 hrs. in dark at 25° C) of the seeds of *Phaseolus vulgaris*, succinic dehydrogenase reaction with TTC method appeares most strongly in apical meristem and procambium, i. e. in the most embryonal cells, and weakly in pith and periderm. On the same stage of germination, however, the hematoxylin staining in fixed preparation and the supravital staining with Janus green B demonstrated that the mitochondria are distributed in pith and periderm but not in apical meristem and procambium. On the other hand, in the late stage of germination, the mitochondria were found in all cells of the tissue. These results revealed that the enzyme found in the cells of procambium of radicle may be organized into cytoplasmic granules, when it is found in the cambial cells in the seedling.

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植物の生活型に関する二, 三の問題点　I[*]

沼　田　　眞[**]　●　浅　野　貞　夫[***]

Makoto NUMATA and Sadao ASANO: Some Considerations Concerning
the Biological Types of Plants. I.

1.　従来の研究と問題点

　植物の生活型に関する研究は数多くあり, とくに今世紀にはいつて Raunkiaer (1907) がその生活型のシステムを確立していらい, これを使用するものが多い。最近までのこの方面の研究状況について Adamson (1939) や Cain (1950)の綜説によつて概況をうかがうことができる。

　生活型システムについては, Raunkiaer の life-form のように種を抽象しようとする線と, Grisebach (1872) の Vegetationsform といつた

種属系統を手がかりとした線，さらに Du Rietz (1931) のように生活型の適応的意義を否定するものなどのいくつかの流れがある。

われわれは少くとも，生活型の適応的意義を認め，これを環境条件の反映としての生活様式の類型とみなす。すなわち生物はその生活環境の諸条件との密接な相互作用のうちに，そうした全体の関連を反映した構造や機能を示すものと考えられるが，そのさい，植物の現在の生活にとつて生態的意義の明らかないくつかの特徴をとらえて類型分類したものが生活型にほかならない。しかも生物主体の側に integration のレベルがあるように，環境の側にもこれに対応したレベルが考えられ，Raunkiaer 式の生活型にもとずいた組成表 (biological spectrum) は，かなりマクロなレベルでの環境を植物主体的に把握しようとしたものとみなしうる。

ところでわが国でも Raunkiaer の生活型システムはつとに用いられてきているが，なかでも，わが国全体の植物気候を論議することを目標とした Horikawa und Sato (1938) の仕事は十分再検討しておく必要がある。Cain (1950) もそのデータを落葉広葉樹林帯に属する地域の一つの代表例としてあげているが，本邦植物の生活型および生活型統計については，未解決のまま放置されている問題がいくつかある。従来の研究にみられる問題点の主なものをあげてみると，

1) わが国のように南北に長く，暖帯から亜寒帯にまでわたつているようなところで，日本という一つの行政区劃的ワクをとつて，そこの自然条件（たとえば気候帯）による層別をせずに，ただ算術的に統計をすることが，生態的にどれだけの意味をもつか。そういう場合の平均（平均値としてあらわされた百分率）とは，生態的にどういう意味をもつのか。この点を明らかにしておかないと，前記のごとく，Cain のような誤つた引用のしかたをもたらす。Raunkiaer の " normal

spectrum " に関しても，これと同様な問題がおこる。

2) 生活型の判定において問題がないかどうか。われわれは 1953 年以来，生活型を一つ一つ現地にのぞみ各季節の観察によつて検討しているが，今まで同一の種について異つた生活型に属するような発表がなされている場合，そこには判定の誤りではないかと思われる場合があること，一方，時には個体ごとにかなりの変動のみられる場合もあること，したがつて相当数の個体を検討する必要があると同時に，斜面など地形的条件による微気候を反映している場合などのあること，を注目している。

3) Boysen-Jensen (1949), Cain (1950) など多くの人によつてすでに指摘されているとおり，"species spectrum" （種類組成表）では植物気候を論議するのに望ましくない場合が多い。広義の" frequency spectrum " （被度による測定などをも含めて）を用いる必要がある。それもとくに植物気候との関連においては，ある地域のクライマックスまたはそれに近い植生がえらばれねばならない。途中相の生活型統計はおのずから別な意味をもつてくる。

4) Raunkiaer の生活型といえども，系統的なものを十分排除しえているわけではないことを注意する必要がある。たとえば単子葉植物に G がおおいといつたことが，種類組成表には大きくひびいてくる。

5) いずれの場合にも，サンプリングの思想はいままではいついていないが，やはりある地域の生活型統計には，層別をしない全数調査ではなく，層別抽出によつてその地域の生態的特色をとらえる方法がとられねばならない。

生活型および生活型統計に関しては，以上のようなさまざまの問題があり，これからそれらの問題を順次に論議してゆきたいと思う。ここではその第 1 報として，2)の生活型判定の問題を，とく

（註）繁殖型（migrule type）は植物のひろがり方（散布や土着）を中心とした生活型の一つで，有性生殖器官の型 D (disseminule type) と栄養生殖器官の型 R (radicoid type) とにわける。それぞれをさらに 5 型に細分するが，これらをごく簡単に説明すると，D は散布様式で，D_1：風散布，水散布，D_2：動物や人間に附着して散布，D_3：自力ではじきとばす，D_4：そのまま重力によつて落下，D_5：原則として種子繁殖をしないもの；R は根糸，匍匐茎，地下茎の型で，R_1〜R_3 根茎植物（R_1 は根茎のひろがりの大きいもの，R_2 は中間，R_3 は小さい——一般に節間が短縮した形），R_4；匍匐茎，5R；栄養系をつくらない（単立性）のもの，詳細は原論文をみて頂きたい。

に繁殖型（migrule type——沼田 1948, Numata 1954）のR_3型（radicoid tipe の一つ）に関して注意すべきいくつかの場合についてのべたい。
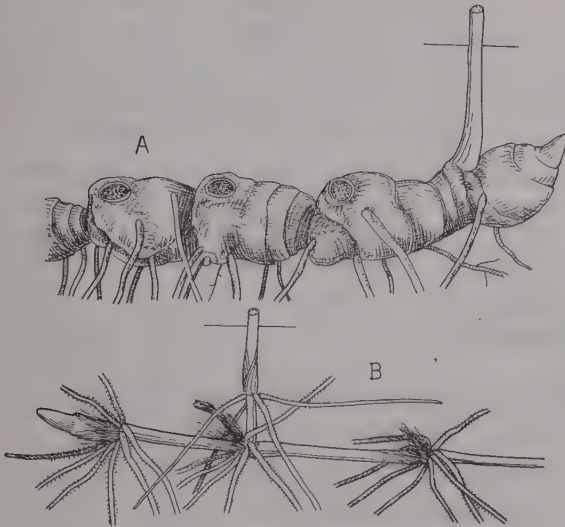
2. 繁殖型の判定に注意すべき場合

生活型の判定上とくに問題を生じやすい場合として，HとGの判別のむずかしい場合とか，繁殖型については根茎植物（R_1〜R_3）のうちとくに節間が短縮して単立的な R_5 と区別しにくい場合などがある。じつさいに地下部をほつてみると，従来の経験などから予想していたのとはかなりちがうことがある。本稿では上記の後者の問題について検討したい。

図A）ユリ科のアマドコロ属 *Polygonatum*（R_3G），ユキザサ属 *Smilacina*（R_3G），ウコギ科のトチバニンジン属 *Pauax*（R_3G）などにみられ，古い地上茎の跡（scar）をもつた短い根茎が塊茎状にふくれて団子状につながる。塊茎状のところからは不定根がで、先端は来年の地上茎の芽，その手前に一本だけ今年の地上茎がでている。堀らないで判定するときR_5としやすい。

図B）ユリ科のツクバネソウ属 *Paris*（R_3G），ラン科のコイチョウラン属 *Ephippianthus*（R_3G）のあるもの，ホトトギス属 *Tricyrtis* とくにヤマホトトギスなどにみられる。これは明瞭な根茎をなすが，地上茎はやはりA）ににて毎年一本しかでないので地上からの判断では R_5 にするおそれがある。地上茎の跡は消えてよくみえない。図の毛状のものは低出葉の葉脈である。

図C）毎年先端部から地上部がでるので図のような結節となり，斜めに地中にはいつた根茎の形をとる。このように地下部がだんだん深いところに進んでいくのは，ある種のロゼット植物の根の成長のしかた（いわゆる root contraction）に似ている。前の年の低出葉が一部のこつている。スミレ科のナガバノスミレサイシン *Viola Bisseti*，スミレサイシン *Viola vaginata*（いずれもR_3H），キツネノボタン科のオウレン属 *Coptis*（R_3H），ラン科のオニノヤガラ属 *Gastrodia*（R_3G）などにみられる。

図D）鱗茎が短い根茎（連絡枝）で水平的にジュズ状（いわゆる moniliform）につながる場合で，ラン科のエビネ属 *Calanthe*，サイハイラン属 *Cremastra*，コケイラン属 *Ororchis*，コクラン属 *Liparis*（いずれも R_3G）などがある。地上茎は簇生するのでR型の判定はむずかしくな
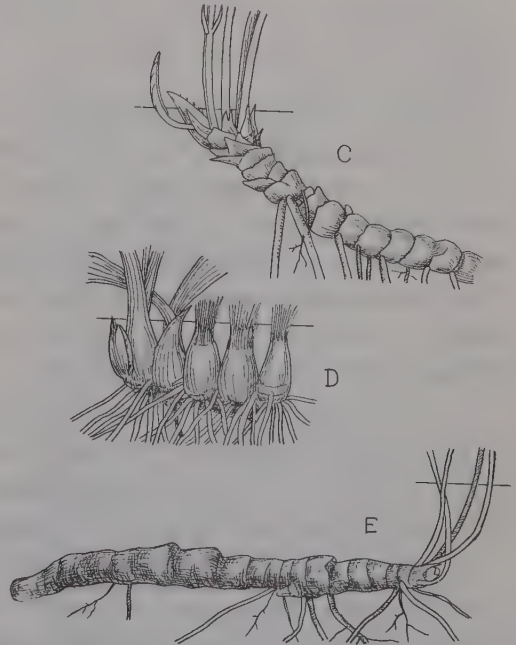
いが，R_3 としてはやはり特殊なタイプである。

図E）C）とにるが，短い節間を有する根茎は横走し，生活型がGとなる点がちがう。キツネノボタン科のイチリンソウ属 *Anemone* など。

図F）この型は各科にわたつてひろくみられ
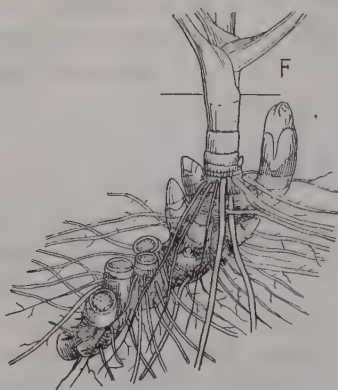
るものであるが，直線的な拡がり方がつよくでること，A）ににて根茎が肥厚したものと考えられるが，斜走し，横から地上茎がでることや，地上茎がかならずしも一本とはかぎらないことなどがA）とことなる。顕著なものの例をあげると，キク科のオケラ属 *Atractylodes* (R₃G)，セリ科のカノツメソウ属 *Spuriopimpinella* (R₃G)，キツネノボタン科のサラシナショウマ属 *Cimicifuga*，レンゲショウマ属 *Anemonopsis* (いずれも R₃G)，ラン科のオサラン属 *Eria* (R₃E) のごときがある。

Summary

1) There are several problems to be discussed in the field survey and statistics of biological types of plants. The important ones among those are as follows:

a. In the case of Japan covering three climatic zones, it will be of little ecological value to collect statistics as a whole unless stratifying those areas.

b. There are some cases difficult to judge a biological type to which a plant belongs. Particularly the individual variation of the highest position of perennial buds and the influences of microclimatic conditions based on topography are to be noticed.

c. The ecological meaning of species spectrum is restricted within narrow limits. The frequency spectrum in a wider sense sholud be used.

d. The concept of phylogentetic type is not fully removed even in the Raunkiaer's life-form system. Abundance of geophytes in the monocotylous plants, for instance, exerts an influence upon the species spectrum.

e. The sampling techniques have not been systematically introduced in the former statistics of biological types. The sampling method will be also useful as in the case of the vegetational analysis.

2) In this report, some considerations concerning the point b. mentioned above will be described. Especially the cases difficult to judge whether R_3 (a type of rhizome plants) or R_5 (non-clonal growth) in the radicoid types proposed by the senior author have been taken up as follows (cf. Fig. A~F):

A) The tuberous enlargements of the rhizome with the conspicuous scars left by the fall of the erect stems of previous seasons. We are apt to judge as R_5 by the only one erect stalk of the current season.e . g. *Polygonatum, Smilacina, Pauax*.

B) A distinct rhizome from which a single erect shoot develops each year and the remnants of similar shoots of former seasons are not clear. e. g. *Paris, Ephippianthus, Tricyrtis*.

C) A obliquely ascending rhizome similar to the lowering of a rosette plant from year to year by the root-contraction. The scale leaves of former seasons

remain partly. e. g. *Viola Bisetti, Viola vaginata, Coptis, Gastrodia.*

D) A beadlike chain of tubers. It is a special form of R_3. e. g. *Calanthe, Cremastra, Ororchis. Liparis.*

E) A horizontal rhizome with short internodes. It is similar to C) besides the character of a geophyte. e. g. *Anemone.*

F) An obliquely asbending rhizome from the sides of which a few erect stems rise. e. g. *Atractylodes, Spuriopimpinella, Cimicifuga, Anemonopsis*

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Georg Tischler' H, D. Wulff: (1953~1956) Angewandte
Pflanzenkaryologie (続刊) の紹介

Handbuch der Pflanzenanatomie (K. Linsbauer, G.·Tischler, u. W. Zimmermann 監修) のBd II として刊行された Allegemeine Pflanzenkaryologie (1921-'22) の補遺として G. Tischler 教授は Angewandte Pflanzenkaryologie を編纂中 1954 年暮にわかに逝去したが，その仕事はその高弟 Heinz Diedrich Wulff 教授によつて継続され 1956 年 1 月第 3 分册が刊行された。引つづき第 4，第 5 分册も本年中に完結刊行の予定である。

Lief, I. 208 Seiten, mit 33 Textabbildungen. 1953. DM 39. Lief. II. 208 Seiten, mit Textabbildungen. 1954, DM 39. Lief. III. 208 Seiten, mit 35 Textabbildungen. 1956. DM 39. Lief. IV. (Schlutz des Ergänzungsbandes) 1956 予定. Lief. V. (Register) 1956 予定.

第 2 分册は 4. Die Genom-Mutationen (209-342), 5. Die Chromosomen-Mutationen (342-400), 6. Die permanenten Hybriden (400-417), 第 3 分册は 7. Die Euhybriden und ihre Sterilitätsphänomene (417-501), 8. Das Burdonen-Problem (501-503), 9. Die Entstehung neuer Arten und Gattungen (504-564), 10. Die Bedeutung der Chromosomenforschung für die Physiologie (564-624) (未了) 含んで

いる。

第 3 分册の内容の一部について簡単に紹介し，大方の育種研究者，遺伝研究者，生物研究者に推薦する。まづ感ずることは斯学における日本人研究者の業績が極めて大きく，それが高く評価されていることであり，また視野が極めて広いことである。「雑種と不稔性」についても広く高等植物から菌類に到るまで数多くの例があげられ，不稔性の問題，雑種強勢の問題が多角的に論じられている。核学的不調和がもたらす異質原形体の生理的異常が胚の発育に正負の影響を与えること，雑種胚の抽出物（主として核的成分）が正常胚に癌組織を誘発する実験（たとえば Satina and Blakeslee 1925~1954: Datura hybrid）など，或は接木雑種において起る代謝系の変化が染色体数を変化せしめうることなど（Brabec 1953, 1954）の興味深いデータから静的な Pflanzenkaryologie が動的なものへ脱皮しつつある状態を示すとともに多くの示唆を与えている。1912年 H. Winkler が *Solanum lycopersicum* (2n=24) と *S. Nigrum* (2n=72) との接木実験でつくり出した雑種（Burdo と命名）*S. Darwinianum* の問題もまた新しい見地から研究し直される命題であるとしている。(Gebrüder Bornträger, Berlin-Nikolassee) (N. T.)

遊離葉緑体における澱粉の
形成について　I

小 野 林* ・ 小 長 光 与 壯*

Hayashi ONO and Yoso KONAGAMITSU: The Formation
of Starch in the Isolated Chloroplast　I

細胞内における chloroplast 中の starch 形成についての観察は多いが，細胞より遊離された chloroplast 中における starch 形成については殆ど報告されてない様に思ふ。又細胞内における phosphorylase の所在に関して従来 chloroplast や leucoplast 等の plastid のみにあるといふ観察 (Yin, 1948 etc.) と，chloroplast になく cytoplasma にのみあるといふ観察 (Stocking, 1952) がある。著者は先に孔辺細胞や大根 tuber 組織の Starch 形成の観察から Phosphorylase は plastid, cytoplasma の何れにも存在しその作用は細胞内の生理的要因の変化で二次的に支配され，又一般に plastid に特にその作用が強いものと考へた。その結果細胞内の条件の変化によつて或時は starch が plastid 中にのみ形成され，或時は cytoplasma 中にのみ形成されるはずである。それで著者等はこれ等二つの異つた観察は細胞内における phosphorylase の存在の場所の問題でなくてその作用の強弱の問題であると考へた。此の考は細胞より遊離された chloroplast や cytoplasma の starch 形成を観察する事によつて確められたのでその結果をかんたんに報告する。

実験材料と方法

自然状態又は暗処理により葉内の starch を消失させた *Raphanus sativus* と *Bryophyllum calycinum* の葉を使用した。starch 形成基質として醋酸緩衝液で pH 6.0 に調製した G-1-P (Coriester) や種々の糖の 0.01 Mol. 溶液を使用した。之等の基質を hole slide glass に入れその中で葉を切り細胞を破壊し chloroplast や cytoplasma を遊離させ，形成された starch を顕微化学的に沃度指薬で検した。又別に葉を pH 6.0 に醋酸緩衝で調製した 0.05 Mol. の Glucose 中において Waring Blendor で homogenate に作り。20〜25mm の脱脂綿層を通過させ濾液を 1800-2000 r. p. m で 4分間遠心分離し chloroplast を遊離させて使用した。之等の操作は 5°〜10°C の低温で出来るだけ速に処理した。

実験結果と考察

1. **概観**. 遊離された chloroplast や cytoplasma では G-1-P 基質からのみ starch が形成されるが，glucose, fructose 又は sucrose より形成されない。Fig. 1 の様に *Bryophyllum* の chloroplast は基質に浸して 0.5 時間後から starch の形成が認められる。starch grain は始め球状で chloroplast 中に散在するが，後には chloroplast 中に充満する。大根の葉より遊離された chloroplast では *Bryophyllum* における様に多くの starch の形成は認められなく大体 3〜9 個の粒が散在するに止る。此の様な G-1-P 基質中における starch の形成はそれが自然状態で形成された starch が暗所においた為に消失する時の逆の経過を辿る。starch は常に chloroplast 中にのみ形成されるものでなく遊離された cytoplasma にも形成され chloroplast の膜に附着するか又は網状構造に並び著しい starch の沃度反応を呈する。 starch が chloroplast にのみ形成され cytoplasma に形成されない場合，その逆の場合，又何れにも一様に強く形成される三つの場合が観

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*九州大学教養部生物学教室

Biological Laboratory, General Education Department, Kyushu University

察された。之等の現象は著者が先に述べた様に phosphorylase が chloroplast と cytoplasma 何れにも存在し細胞内の生理的条件の変化によつて phosphorylase の作用力が支配される事を証明する。即ち chloroplast や cytoplasma の phosphory'ase の starch 形成作用力はそれ等が細胞から遊離される前の細胞内における時の条件, 例えば光, 温度等の要因に支配され, その時に決定された作用力は, それ等が細胞から遊離された後にも現れる為に, 遊離された後に与えた条件の変化

plasma の中の phosphorylase の活性度は細胞内に存在する時に細胞内の生理的条件の変化によつて決定される。大根の葉より遊離された cytoplasma には, *Bryophyllum* よりも starch がよく形成され又比較的低温においても starch が形成される。

3. starch 形成に及ぼす水素イオン濃度の影響: *Bryophyllum* の葉から遊離された chloroplast には 3.0～8.0 の比較的広い範囲の pH 域値において starch が形成されるが, 大根葉より遊

Table 1.　The effect of temperature on the formation of starch in the isolated chloroplast and cytoplasma(In these cases starch was not formed in the water that contains no G–1–P)

Material plants	Temperature in wh. ch plants were treated for 7 days before chloroplast and cytoplasma isolating	Temperature in which the isolatee chlorodlast and cytoplasma were treated in G–1–P substrate	Activity of starch formation	
			Chloroplast	Cytoplasma
Bryophyllum	0° C.	0° C. 20° C.	± 卄	— ±
	20° C.	0° C. 20° C.	卅 卅	— ±
Raphanus	5°–10° C.	5° C. 20° C.	± ±	± 卄
	20° C.	5° C. 20° C.	+ +	+ +

の影響は余りうけない。

2. starch 形成に及ぼす温度の影響: 前以て 20°C の高温に処理した植物の葉から遊離された chloroplast は 0°C の低温におかれた葉から遊離された場合よりも starch がよく形成される (Table 1)。chloroplast や cytoplasma が遊離された後の温度, 即ち G–1–P 基質中における温度の影響を余りうけない。此の現象は著者が[6] 先の報告で示し孔辺細胞の starch 形成における場合と同様である。

此の結果は与えた温度の変化は直接 phosphorylase の作用に影響する事は少く, 細胞内条件の変化に影響を及ぼし, 遊離された chloroplast 又は cytoplasma の phosphorylase 作用が間接に支配される事を示す。即ち chloroplast や cyto-

離された chloroplast では pH 4.5～6.3 に形成された。一般に pH 5.0～6.3 によく starch が形成される。これは前報[5] で述べた様に孔辺細胞や組織細胞の starch 形成, 馬鈴薯や大根 tuber より分離された phosphorylase の *in vitro* における作用の場合と同様である。*Bryophyllum* では大根より遊離された物より比較的酸性において starch が形成されるのは前者の 酸植物としての特性による物と思ふ。

4. starch 形成に及ぼす光の影響: 遊離された chloroplast や cytoplasma に G–1–P 基質中で starch が形成される時には光は影響しない。然し chloroplast や cytoplasma を植物の葉から遊離させる前に葉のうける光の影響は大きく, その影響はそれが遊離された後の starch 形

Table 2. The formation of starch in the chloroplast and cytoplasma isolated from the leaves of *Raphanus* kept in the light and in the dark.

The condition in which leaves was treated before isolating of chloroplast and cyto-plasma	The activity of starch formation	
	Chloroplast	Cytoplasma
In the light ·································	卅	卅
In the dark, 7 days. ·····················	±	+
In the dark, more than 10 days.	—	—
In the dark 7 days and then exposured to the light before isolating chloroplast and cytoplasma ·························	+	卌

Fig. 1. Schematic figures of starch formed in isolated chloroplast.

Raphanus:

At beginning　　　　After 12 hours

Bryophyllum:

At beginning　　　　After 30 minutes

After 1 hour　　　　After 2 hours
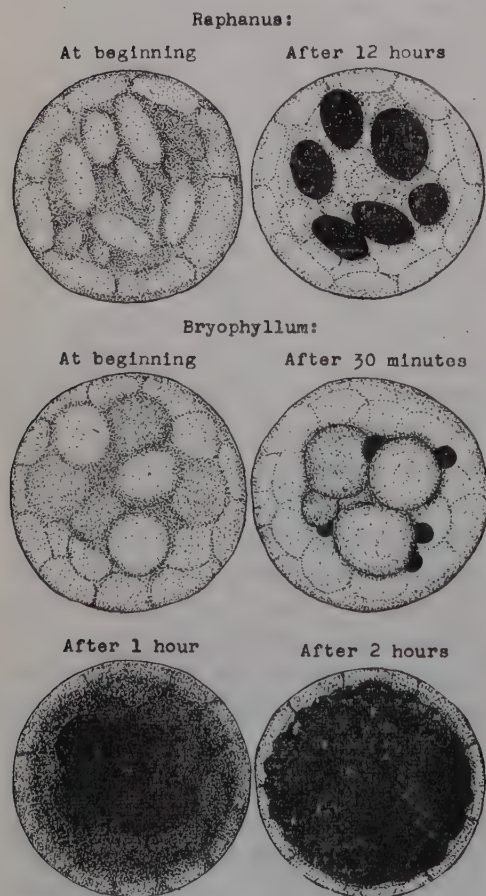
成において強く現れる。則ち Table 2 に示した様に暗所よりも明条件においた葉から遊離された chloroplast や cytoplasma 中により多くの starch が形成された。暗所におかれた葉より遊離された場合には chloroplast よりも cytoplasma により多くの starch が形成され phosporylase の活性度が大である。長く暗処理した植物より遊離した chloroplast や cytoplasma に starch は形成されないが，此の性質は植物の種類によつて異る。例えば *Bryophyllum* は大根に比較して比較的永く暗所においたものでも starch がよく形成される。

暗処理して starch を形成しなくなつた葉も chloroplast や cytoplasma を遊離させる前に葉を再び短時間でも露光すると，それより遊離された chloroplast や cytoplasma の starch の形成と phosphorylase の作用力は著しく増大する。此の現象は外的条件の変化によつて支配される細胞内の生理的条件の変化が chloroplast や cytoplasma の中における phosphorylase の活性度を支配し，その作用が chloroplast や cytoplasma を遊離させた後にも現れるためである。此の場合短時間の露光では chloroplast よりも cytoplasma に starch 形成作用が大となる。

結　論

上述の様に G-1-P を基質として，細胞内においては勿論の事，細胞より遊離された chloroplast や cytoplasma に starch が形成され，それ等における phosphorylase の作用力は，それ等が細胞から遊離される前の外的条件の変化により起る細胞内条件の二次的影響によつて支配される事を明にした。その結果 phosphorylase は chl-

oroplast, cytoplasma 何れにも存在し，その作用の発現に差異が起る物である。即ち高温，光条件に処理すると，低温，暗条件に処理した葉から遊離した物よりも starch がよく形成される。暗所に永くおいた葉より遊離された chloroplast や cytoplasma には starch が形成されない。然し此の場合 chloroplast や cytoplasma を遊離さ

せる前にたとえ短時間でも再び暗所から明所に処理すると遊離された条件においても著しく starch 形成作用は増大する。遊離された chloroplast や cytoplasma における starch 形成に適当な水素イオン濃度は pH 5.0〜6.0 であり，孔辺細胞や *in vitro* における phosporylase の作用に及ぼす影響と同様である。

Summary

1. In the chloroplast and cytoplasma isolated from the leaves of *Bryophyllun* and *Raphanus*, starch was formed mere in the G–1–P substrate.

2. The formation of starch in the isolated chlroplast and cytoplasma were controlled by the change of physiological factors' which has an effect before they are isolating.

3. More starch was formed in the chloroplast and cytoplasma isolated from the leaves kept in the light and in higher temperature than in those from the leaves kept in the dark and in lower temperature.

4. Starch forming activity diminishes earlier in chloroplast than in cytoplasma when the leaves kept in the dark.

5. Starch is easily formed in the chloroplast and cytoplasma isolated from the leaves kept in the dark for many days but exposed again to the light immediately before isolation.

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ノコンギク属植物の核型分析　IV*

藤　原　悠　紀　雄**

Yukio HUZIWARA: Karyotype Analysis in *Aster* IV

筆者[1,2,3] は前 3 報において *Aster* 属 14 種，6 亜種および 3 変種について核型を明らかにした。今回さらに 5 種，1 亜種および 4 変種について核型分析を行つたので報告する。

材料および方法

観察の方法は前回と同じであつて用いた材料は次の通りである。

Aster dimorphophyllus Fr. et Sav.　タテヤマギク

A. maackii Regel　ヒゴシオン

A. ageratoides Turcz. subsp. *leiophyllus* Kitamura　シロヨメナ

A. ageratoides Turcz. subsp. *leiophyllus* Kitamura var. *sawadanus* Kitamura　キントキシロヨメナ

A. ageratoides Turcz. subsp. *ovatus* Kitamura var. *littoricola* Kitamura　ハマコンギク

A. ageratoides Turcz. subsp. *ovatus* Kitamura var. *hortensis* Kitamura　コンギク

A. ageratoides Turcz. subsp. *ovatus* Kitamura var. *humilis* Kitamura　コマチギク

A. dubius Onno　アヅマギク

A. ptarmicoides T. et G.　テリアツバギク

A. novi-belgii L.　ユウゼンギク

結　　果

1.　タテヤマギク *Aster dimorphoyhpllus* Fr. et Sav. $2n=18$　箱根上二子産　(figs. 1, 2, 21; table I)

体細胞染色体 18 個は大きさと形とから 8 種類に区別される。着系点は 2 対 (5, 6 ; 17, 18) が median 他はすべて submedian である。最大の染色体 1 対 (1, 2) は L^2E 染色体であつて短腕に二次狭窄をもち長さ 7.2μ あり，最小の 1 対は長さ 4.8μ である。葉が深裂する種類 (var. *divisus* Makino) と葉が卵円形の種類 (var. *indivisus* Makino) とにおいて核型の差異は認められない。

核型は次の式で表わされる。

$$K(2n)=18=2^{cs}A_1^{sm}+2A_2^{sm}+2A_3^{m}+4B^{sm}$$
$$+2C_1^{st}+2C_2^{cm}+2D^{sm}+2E^{m}$$

Table I.　Measurements of somatic chromosomes in *A. dimorphophyllus*

Chromosomes	Length in μ	Centromere
1, 2	7.2=4.2+1.8+1.2	sm
3, 4	7.2=4.2+3.0	sm
5, 6	7.2=3.6+3.6	m
7-10	6.6=3.6+3.0	sm
11, 12	6.0=4.2+1.8	s t
13, 14	6.0=3.6+2.4	sm
15, 16	5.4=3.0+2.4	sm
17, 18	4.8=2.4+2.4	m

2.　ヒゴシオン *A. maackii* Regel $2n=18$ 阿蘇山産　(figs. 3, 4, 22; table II)

本種は阿蘇山附近にのみ生育する稀産種である。体細胞染色体 18 個は 6 種類に区別できる。着糸点は 2 対 (11, 12 ; 15, 16) が median, 他はすべて submedian である。最大の染色体 1 対 (1, 2) は L^2E 染色体であつて短腕に二次狭窄をもち長さ 6.6μ あり，最小の染色体は長さ 4.2μ である。

核型は次の式で表わされる。

$$K(2n)=18=2^{cs}A^{sm}+8B_1^{sm}+2F_2^{m}+2C^{sm}$$
$$+2D^{m}+2E^{sm}$$

Figs. 1–20. Somatic chromosomes of *Aster*. × 1200.

1–2) *A. dimorphophyllus* 2*n*=18.   3–4) *A. maackii* 2*n*=18.   5–6) *A. ageratoides* subsp. *leiophyllus* 2*n*=18.   7–8) *A. ageratoides* subsp. *leiophyllus* var. *sawadanus* 2*n*=36. 9–11) *A. ageratoides* subsp. *ovatus* var. *littoricola* 2*n*=36.   11–12) *A. ageratoides* subsp. *ovatus* var. *hortensis* 2*n*=36.   13–14) *A. ageratoides* subsp. *ovatus* var. *humilis* 2*n*=36. 15–16) *A. dubius* 2*n*=18.   17–18) *A. ptarmicoides* 2*n*=18.   19–20) *A. novi-belgii* 2*n*=48.

Table II. Measurements of somatic chromosomes in *A maackii*

Chromosomes	Length in μ	Centromere
1, 2	6.6=3.6+1.8+1.2	sm
3-10	6.0=3.6+2.4	sm
11, 12	6.0=3.0+3.0	m
13, 14	5.4=3.0+2.4	sm
15, 16	4.8=2.4+2.4	m
17, 18	4.2=2.4+1.8	sm

3. シロヨメナ *A. ageratoides* Turcz. subsp. *leiophyllus* Kitamura 2n=18 妙高産 (figs. 5, 6; table III)

本植物は染色体数の変化が著しく，2n=18 の2倍種のほか高田市産のものに 2n=36(fig. 23) および 2n=38，箱根上二子産のものに 2n=54 が認められた。核型分析は2n=18のものについて行つた。体細胞染色体18個は 8 種類に区別される。着糸点は 2 対 (7, 8；9, 10) が median，他はすべて submedian である。最大の染色体 1 対(1, 2) はL2E 染色体であつて長さ 7.8 μ，最小の染色体は長さ3.8 μ である。中位の染色体 1 対 (11, 12) においても短腕に二次狭窄が認められる。

核型は次の式で表わされる。

$$K(2n)=18=2^{cs}A^{sm}+2B^{sm}+2C_1^{sm}+4C_2^{m}$$
$$+2^{cs}D^{sm}+2E^{sm}+2F^{sm}+2G^{sm}$$

Table III. Measurements of somatic chromosomes in *A. ageratoides* subsp. *leiophyllus*

Chromosomes	Length in μ	Centromere
1, 2	7.8=4.2+2.4+1.2	sm
3, 4	6.6=3.6+3.0	sm
5, 6	6.0=3.6+2.4	sm
7-10	6.0=3.0+3.0	m
11, 12	5.4=3.0+1.8+0.6	sm
13, 14	4.8=3.0+1.8	sm
15, 16	4.2=2.4+1.8	sm
17, 18	3.8=2.4+1.4	sm

4. キントキシロヨメナ　*A. ageratoides* Turcz. subsp. *leiophyllus* Kitamura var. *sawadanus* Kitamura 2n=36　箱根金時山産 (figs. 7, 8; table IV)

本植物においても 2n=36 および 2n=38 が認められ，核型分析は 2n=36 の株について行つた。36 個の染色体は 10 種類に区別できる。最大の染色体は長さ 7.8 μ あり，最小の染色体は長さ 3, 6 μ である。L2E 染色体は 1 対認められる。着糸点は 6 対 (17, 18：19, 20；21, 22；23, 24；31, 32；35, 36) が median，他は submedian である。

核型は次の式で表わされる。

$$K(2n)=36=2^{cs}A^{sm}+6B_1^{sm}+2B_2^{sm}+6C_1^{sm}$$
$$+8C_2^{m}+2D^{sm}+4E_1^{sm}+2E_2^{m}+2F^{sm}+2G^{m}$$

Table IV. Measurements of somatic chromosomes in A. *ageratoides* subsp. *leiophyllus* var. *sawadanus*

Chromosomes	Length in μ	Centromere
1, 2	7.8=4.2+2.4+1.2	sm
3-8	6.6=4.2+2.4	sm
9, 10	6.6=3.6+3.0	sm
11-16	6.0=3.6+2.4	sm
17-24	6.0=3.0+3.0	m
25, 26	5.4=3.0+2.4	sm
27-30	4.8=3.0+1.8	sm
31, 32	4.8=2.4+2.4	m
33, 34	4.2=2.4+1.8	sm
35, 36	3.6=1.8+1.8	m

5. ハマコンギク　*A. ageratoides* Turcz. snbsp. *ovatus* Kitamura var. *littoricola* Kitamura 2n=36　伊豆大島産 (figs. 9, 10, 24; table V)

体細胞染色体 36 個は 10 種類に区別できる。最大の染色体は長さ 7.8 μ 最小の染色体は長さ 2.4 μ であるから染色体間の長さの差が著しい。L2E 染色体は 1 対認められる。着糸点は 3 対(17, 18；19, 20；35, 36)が median，他は submedian である。

核型は次の式で表わされる。

$$K(2n)=36=2^{cs}A^{sm}+4B^{sm}+2C^{sm}+8D^{sm}$$
$$+2E^{m}+2F_1^{m}+4F_2^{sm}+8G^{sm}+2H^{sm}+2I^{m}$$

Table V. Measurements of somatic chromosomes in *A. ageratoides* subsp. *ovatus* var. *littoricola*

Chromosomes	Length in μ	Centromere
1, 2	7.8＝4.2＋2.4＋1.2	sm
3-6	6 6＝4.2＋2.4	sm
7, 8	6.0＝3.6＋2.4	sm
9-16	5.4＝3.0＋2.4	sm
17, 18	4.8＝2.4＋2.4	m
19, 20	3.6＝1.8＋1.8	m
21-24	3.6＝2.4＋1.2	sm
25-32	3.0＝1.8＋1.2	sm
33, 34	2.8＝1.8＋1.0	sm
35, 36	2.4＝1.2＋1.2	m

6.　コンギク　*A. ageratoides* Turcz. subsp. *ovatus* Kitamura var. *hortensis* Kitamura 2n＝36 栽培品　(figs. 11, 12; table VI)

体細胞染色体36個は11種類に区別できる。最大の染色体は8.4μ, 最小の染色体は2.4μで長さの差が著しい。L²E 染色体は1対認められる。着糸点は4対 (7, 8; 9, 10; 25, 26; 35, 36) が median, 他は submedian である。

核型は次の式で表わされる。

$$K(2n)=36=2^{cs}A^{sm}+2B^{sm}+2C^{sm}+4D^{m}$$
$$+2E_1^{sm}+6F_2^{sm}+2F^{sm}+4G_1^{sm}+2G_2^{m}+$$
$$8H^{sm}+2I^{m}$$

Table. VI. Measurements of somatic chromosomes in *A. ageratoides* subsp. *ovatus* var. *hortensis*

Chromosomes	Length in μ	Centromere
1, 2	8.4＝4.8＋2.4＋1.2	sm
3, 4	7.2＝4.8＋2.4	sm
5, 6	6.6＝3.6＋3.0	sm
7-10	6.0＝3.0＋3.0	m
11, 12	5.4＝3.6＋1.8	sm
13-18	5.4＝3.0＋2.4	sm
19, 20	4.2＝2.4＋1.8	sm
21-24	3.6＝2.4＋1.2	sm
25, 26	3.6＝1.8＋1.8	m
27-34	3.0＝1.8＋1.2	sm
35, 36	2.4＝1.2＋1.2	m

7.　コマチギク　*A. ageratoides* Turcz. subsp. *ovatus* Kitamura var. *humilis* Kitamura 2n＝36 栽培品　(figs. 13, 14; table VII)

体細胞染色体 36 個は 11 種類に区別できる。

最大の染色体は長さ 7.2μ, 最小の染色体は長さ 1.2μ で染色体間の長さの差が特に著しい。 L²E 染色体は1対認められる。着糸点は6対 (15, 16: 17, 18; 27, 28; 29, 30; 31, 32; 35, 36) が median, 他は submedian である。

核型は次の式で表わされる。

$$K(2n)=36=2^{cs}A^{sm}+2B^{sm}+4C^{sm}+6D^{sm}$$
$$+4E^{m}+2F^{sm}+4G^{sm}+2H_1^{sm}+6H_2^{m}+2I^{sm}$$
$$+2J^{m}$$

Table VII. Measurements of somatic chromosomes in *A. ageratoides* subsp. *ovatus* var. *humilis*

Chromosomes	Length in μ	Centromere
1, 2	7.2＝4.2＋1.8＋1.2	sm
3, 4	6.6＝3.6＋3.0	sm
5-8	6.0＝3.6＋2.4	sm
9-14	5.4＝3.0＋2.4	sm
15-18	4.8＝2.4＋2.4	m
19, 20	3.6＝2.4＋1.2	sm
21-24	3.0＝1.8＋1.2	sm
25, 26	2.4＝1.4＋1.0	sm
27-32	2.4＝1.2＋1.2	m
33, 34	1.8＝1.2＋0.6	sm
35, 36	1.2＝0.6＋0.6	m

8.　アヅマギク　*A. dubius* Onno　2n＝18 岩手玉山産　(figs. 15, 16, 25)

本種の核型はミヤマアヅマギクのそれと全く一致し次の式で表わされる。

$$K(2n)=18=2A^{st}+2B^{st}+2B_2^{sm}+2C^{st}+$$
$$2D^{st}+4E_1^{sm}+2E_2^{m}+2F^{sm}$$

9.　テリアツバギク　*A. ptarmicoides* T. et G. 2n＝18　栽培品　(figs. 17, 18: table VIII)

本種は北アメリカ原産のものでその染色体は小さく最大 4.2μ, 最小 1.8μ である。18個の染色体は5種類に区別でき, 最大の染色体1対 (1, 2) は長腕に二次狭窄をもつているが, 大きさと形とから見て日本産 *Aster* に見られる L²E染色体とは起原を異にするものと思われる。着糸点は median のものなく, 3 対 (3, 4; 11, 12; 13, 14) が subterminal で, 他はすべて submedian である。

核型は次の式で表はされる。

$$K(2n)=18=2_{cs}A^{sm}+2B_1^{st}+6B_2^{sm}+4C^{st}$$
$$+4D^{sm}$$

Table VIII. Measurements of somatic chromosomes in *A. ptarmicoides*

Chromo-somes	Length in μ	Centro-mere
1,2	4.2＝1.8＋1.2＋1.2	sm
3,4	3.0＝2.4＋0.6	st
5-10	3.0＝1.8＋1.2	sm
11-14	2.4＝1.8＋0.6	st
15-18	1.8＝1.2＋0.6	sm

10.　ユウゼンギク　*A. novi-belgii* L.　$2n=48$
栽培品 (figs. 19, 20, 26: table IX)

本種も北アメリカ原産のものであるが，園芸植物として広く栽培されている。外部形態において変化多く，染色体数も種々である。今回用いた株は $2n=48$ のものである。本種の染色体は極端に小さく，その長さは最大 3.6 μ，最小 1.4 μ，平均 2.0 μ であつて *Aster* 中最小である。着糸点は median のものなく，8 対 (5, 6；7, 8；9, 10；11, 12；13, 14；15, 16；17, 18；19, 20) が subterminal 他は submedian である。二次狭窄をもつた染色体は認められない。

核型は次の式で表わされる。

$$K(2n)=48=4A^{sm}+4B^{st}+12C^{st}+24D^{sm}+4E^{sm}$$

Table IX. Measurements of somatic chromosomes in *A. novi-belgii*

Chromo-somes	Length in μ	Centro-mere
1-4	3.6＝2.4＋1.2	sm
5-8	2.4＝1.8＋0.6	st
9-20	2.0＝1.4＋0.6	st
21-44	1.8＝1.2＋0.6	sm
45-48	1.4＝0.8＋0.6	sm

考察と結論

タテヤマギク，ヒゴシオンおよびシロヨメナの 2 倍種はいずれも 1 対の L^2E 染色体をもつている。4 倍種のキントキシロヨメナ，ハマコンギク，コンギクおよびコマチギクにおいても 1 対ずつL^2E 染色体が認められ，染色体が 4 個ずつ同形の 9 組とはならないことから，これらの 4 倍性植物はすべて異質 4 倍体と考えられる。キントキ
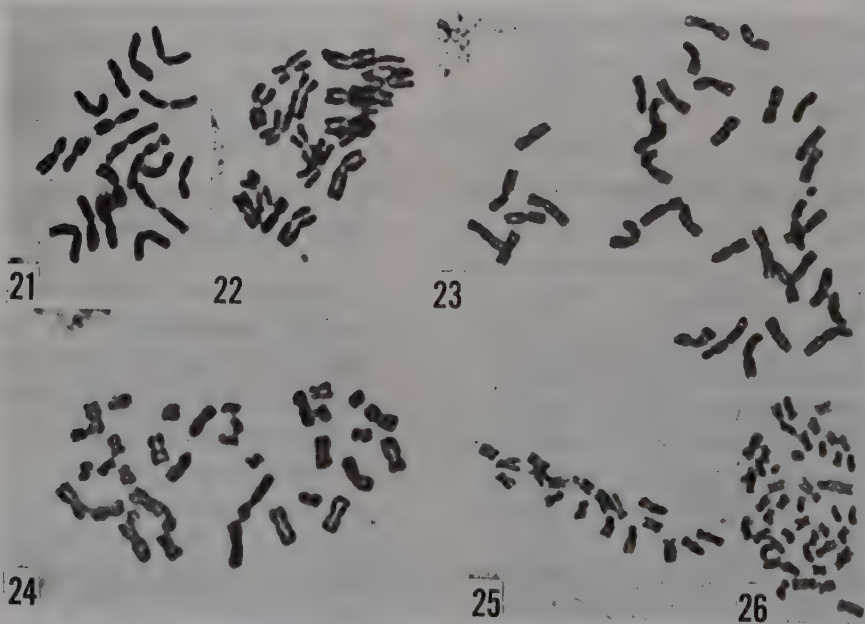
Figs. 21-26. Photomicrographs of somatic chromosomes.　21) *A. dimorphophyllus* $2n=18$.
22) *A. maackii* $2n=18$.　23) *A. ageratoides* subsp. *leiophyllus* $2n=36$.　24) *A. ageratoides* subsp. *ovatus* var. *littoricola* $2n=36$.　25) *A. dubius* $2n=18$.　26) *A. novi-belgii* $2n=48$.

シロヨメナは他の4倍種とは核型がやゝ異り，最大染色体と最小染色体との大きさの差が比較的少い。同一の亜種に属し，互に極めて近縁の変種と考えられるハマコンギク，コンギクおよびコマチギクの間においても核型は全くは一致せず，僅かずつの差異が認められる。

シロヨメナにおいて亜種内倍数性が認められることはキントキシロヨメナ，ハマコンギク，コンギク，コマチギクなど多くの4倍性植物の見られることと共に Aster 属の種の形成にあたつて倍数性が重要なる役割をもつことを示すものであり，また4倍種の多くにおいて染色体間に大きさの差が著しいことは交雑および染色体の構造変化がこの属の進化に関与したことを示すものである。

アヅマギクはその変種であるミヤマアヅマギクと外部形態および生育地を異にするが核型においては全く差異がない。筆者[4] は Aster 属と近縁の Erigeron 属において核型分析を行い，Erigeron 属の染色体が小形で非対称であることを明らかにした。即ちアヅマギクおよびミヤマアヅマギクの染色体が小形で非対称であつて Erigeron 属と核型が類似していることは Aster 属の Alpigenia 節をむしろ Erigeron 属に入れるのが適当であることを示すものである。

北アメリカ種の A. ptarmicoides および A. novi-belgii が既報の A. novae-angliae および A. subulatus と同じく染色体が極端に小形で日本産の Aster と核型が根本的に異ることはアジア種と新世界種とが全く異つた起原をもつことを示すものである。

御指導を賜つている広島大学下斗米教授並びに分類学上の御教示をいただいた京都大学北村教授に御礼申上げる。材料の採集に協力下さつた菊地政雄，井上覚，松浦茂寿の諸氏に対し感謝の意を表す。

Summary

1. The karyotypes of 5 species, 1 subspecies and 4 varieties of *Aster* are reported.
2. The karyotype formulae are as follows:

A. dimorphophyllus

$$K(2n)=18=2^{cs}\ A_1^{sm}+2A_2^{sm}+2A_3^{m}+4B^{sm}+2C_1^{st}+2C_2^{sm}+2D^{sm}+2E^{m}$$

A. maackii

$$K(2n)=18=2^{cs}\ A^{sm}+8B_1^{sm}+2B_2^{m}+2C^{sm}+2D^{m}+2E^{sm}$$

A. ageratoides subsp. *leiophyllus*

$$K(2n)=18=2^{cs}\ A^{sm}+2B^{sm}+2C_1^{sm}+4C_2^{m}+2^{cs}\ D^{sm}+2E^{sm}+2F^{sm}+2G^{sm}$$

A. ageratoides subsp. *leiophyllus* var. *sawadanus*

$$K(2n)=36=2^{cs}\ A^{sm}+6E_1^{sm}+2B_2^{sm}+6C_1^{sm}+8C_2^{m}+2D^{sm}+4E_1^{sm}+2E_2^{m}+2F^{sm}+2G^{m}$$

A. ageratoides subsp. *ovatus* var. *littoricola*

$$K(2n)=36=2^{3s}A^{sm}+4B^{sm}+2C^{sm}+8D^{sm}+2E^{m}+2F_1^{m}+4F_2^{sm}+8G^{sm}+2H^{sm}+2I^{m}$$

A. agertoides subsp. *ovatus* var. *hortensis*

$$K(2n)=36=2^{cs}\ A^{sm}+2B^{sm}+2C^{sm}+4D^{m}+2E_1^{sm}+6E_2^{sm}+2F^{sm}+4G_1^{sm}+2G_2^{m}+8H^{sm}+2I^{m}$$

A. ageratoides subsp. *ovatus* var. *humilis*

$$K(2n)=36=2^{cs}\ A^{sm}+2B^{sm}+4C^{sm}+6D^{sm}+4E^{m}+2F^{sm}+4G^{sm}+2H_1^{sm}+6H_2^{m}+2I^{sm}+2J^{m}$$

A. dubius

$$K(2n)=18=2A^{st}+2E_1^{st}+2B_2^{sm}+2C^{st}+2D^{st}+4E_1^{sm}+2E_2^{m}+2F^{sm}$$

A. ptarmicoides

$$K(2n)=18=2_{cs}\ A^{sm}+2B_1^{st}+6B_2^{sm}+4C^{st}+4D^{sm}$$

A. novi-belgii

$$K(2n)=48=4A^{sm}+4B^{st}+12C^{st}+24D^{sm}+4E^{sm}$$

引　用　文　献

1) 藤原悠紀雄　植雑　66:262 (1953)　　2) ————　同上　97:184 (1954)
3) ————　同上　68:98 (1955)　　4) ————　染色体　25-26:923 (1955)

蘚 類 数 種 の 染 色 体
XI. ハリガネゴケ科の蘚の染色体

矢 野 孝 二*

Koji YANO: On the Chromosomes in Some Mosses.
XI. Chromosomes in Bryaceae-mosses.

1956 年 1 月 13 日受付

筆者は既にハリガネゴケ科のヘチマゴケ属 (*Pohlia*), カサゴケ属 (*Rhodobryum*) の数種について染色体の報告を行つた（矢野 1951, 53a）。亦本科のハリガネゴケ属 (*Bryum*) の染色体数については他の研究者によつて多くの報告がなされている (Él. & Ém. Marchal 1911, Ém. Marchal 1920**. Wettstein 1924, Heitz 1928, Jachimsky 1932, Griesinger 1937**, Wettstein & Straub 1942, 三宮 1955)。然し其他の属については未だ染色体の報告がなく，亦本科の核型については全然報告を見ない。筆者は今回本科の 6 属 20 種につき染色体の観察を行い，それ等の核型を定め得たので，その結果をこゝに報告する。

本研究に用いられた蘚の種名，採集地は次の如くであり，固定並びに染色法は前報告の場合と同様である。

種　名	採集地
Pohlia scabridens (Mitt.) Broth.	越後：高田市
P. revolvens (Card.) Nog.	信濃：八ケ岳
P. columbica (kindb.) Broth.	越後：妙高山
P. cornea (L.) Limpr.	越後：妙高山
P. Wahlenbergii (Web. et Mohr.) Andrews.	越後：妙高山, 黒姫山
P. revoluta (Card.) Ochi.	越後：関山村
P. acuminata Hoppe et Hornsh.	越後：妙高山
P. Suzukii Ochi.	越後：火打山
P. nutans Schreb.	信濃：黒姫山, 越後：妙高山
P. patentissima Ochi.	越後：小滝村
Bryum nagasakense Broth.	越後：高田市
B. argenteum L.	越後：高田市
B. cyclophyllum (Schwagr.) Br eur.	越後：高田市
B. pseudo-alpinum Ren. et Card.	越後：根知村
B. caespiticum L.	越後：火打山
Anomobryum japonicum Broth.	越後：妙高山
Plagiobryum japonicum Nog.	信濃：八ケ岳
Brachymenium exile (Doz. et Molk.) v. d. B. et Lac.	越後：高田市
Rhodobryum roseum (Weis.) Limpr.	信濃：戸隠山
R. giganteum (Hook.) Par.	越後：小出町

観　察

I. *Pohlia*

Pohlia scabridens. (Fig. I, a-d)

雌雄異株，研究に用いられた配偶体の性別は不詳であつた。核型 K(n)=10=V(H)+2J+6(2v+4j)+m(h)。即ち本種の染色体組は 3 個の大きな染色体 (V. 2J), 6 個の小型染色体（およそ 2v, 4j) 及び 1 個の m より成る。最大の V 及び m はそれぞれ異質染色体 H, h であり，それらの形態は既報の多くの蘚の H, h とほゞ共通である。即ち H は median 着糸点を有し一腕（左腕）の尾部に二次狭窄が見られ，亦時に右腕の中央部にも微弱な二次狭窄が認められる。その異常攣縮を示す部分は右腕の尾部約半分と左腕の尾部の小部分である。h はその全部が heteropycnotic であり，且これは Nukleolinus-Chromosomen である。尚これ等の H 及び h の異常攣縮を示す部分は屢々中期に於て染色性が弱く，他の部分と明瞭に区別された。

* 新潟大学教育学部高田分校生物学教室 (Biological Institute, Takada Campus. Faculty of Education, Niigata Untiversity, Takada, Japan).
** Tischler (1927, '38) 参照。

Fig.   I
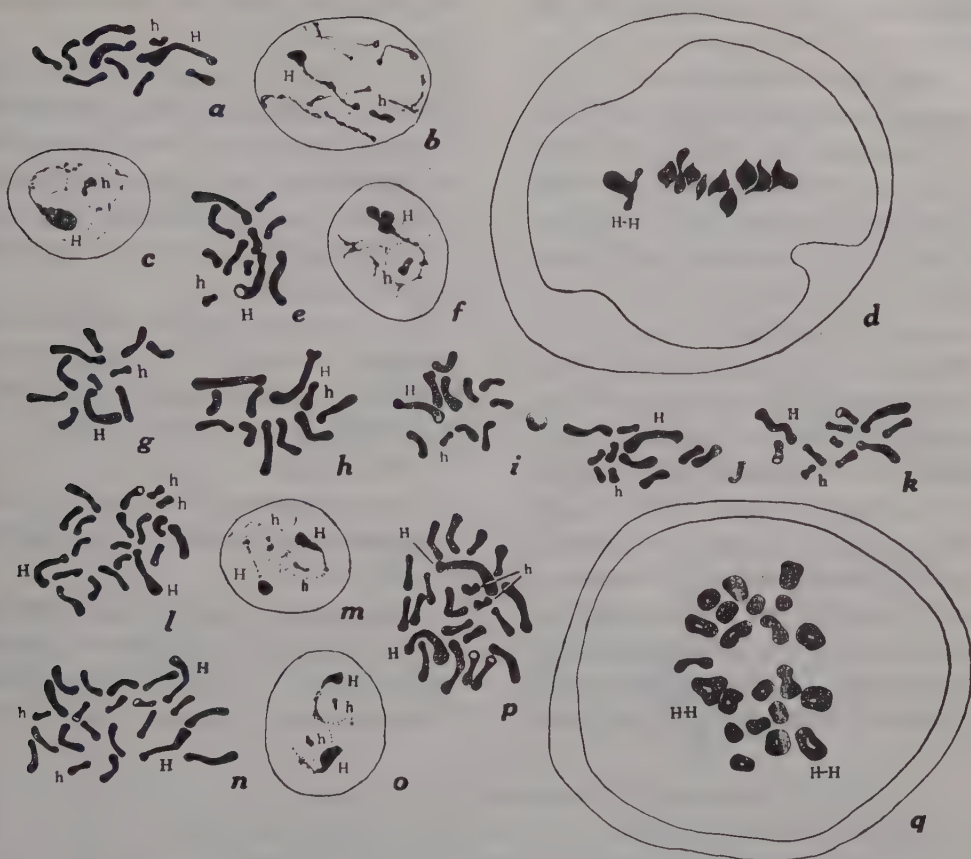
Fig. I. Chromosomes and heteropycnosis in ten *Pohlia*-species. a-d. *P. scabridens*: a, b, c.
gametophyte; d. meiotic chromosomes at MI of SMC; $n=10$.
e, f. *P. revolvens*. g, *P. columbica*. h. *P. cornea*. i. *P. Wahlenbergii*. j. *P. revoluta*. k. *P. acuminata*. e-k. gametophyte, $n=11$. l, m. *P. Suzukii*, gametophyte; $n=20$.
n, o, q. *P.nutans*: n, o. gametophyte; q. meiotic chromosomes at MI of SMC; $n=22$.
p. *P. patentissima*, gametophyte; $n=22$. × 3000

胞子母細胞の減数分裂に於ては10個の bivalent が明瞭であり，これ等のうちに不等対は認められなかつた。尚これ等のうち最大のものは H–H の対合と推定され，これは構成染色体がその一方の腕のみによつて接合している為他の bivalent と異つた独特の形態を示す (Fig. I, d)。

Pohlia revolvens, *P. columbica*, *P. cornea*, *P. Wahlenbergii*, *P. revoluta*, *P. acuminata* (Fig. I, e-k)。

これ等6種は何れも雌雄異株，併し研究に用いられた配偶体の性別は不詳。各種共 $n=11$ であつて上記 *P. scabridens* ($n=10$) よりも1個多い。

筆者は既にこれ等と同様 $n=11$ の染色体数を *P. Fauliei*, *P. elongata* の両種で算定し，核型を V $(H)+6J+3+m(h)$ と報告している。依つて，本属には $n=10$ 及び $n=11$ の2系統があるわけである。今回観察した6種の各染色体組も前記 *P. scabridens* の場合と同様に大小二群の染色体に大別され，何れも次の如き核型式で示される。$K(n)=11=V(H)+2J+7(2v+5j)+m(h)$，即ちこれ等6種の染色体組は異質染色体 (H, h) 及び大型の常染色体 (2J) は *P. scabridens* と同じ形態のものより成るが，小型の j 型常染色体が後者より1個多い。この過剰の j は殊に微小であつ

て，その大きさはほぼmと同様である。従つてこ
れ等の各種は P. scabridens に比すれば高一倍種
に相当する。

Pohlia Suzukii. (Fig. I, l, m)

雌雄同株。本種の染色体数は $n=20$ で，先記
の P. scabridens の二倍種である。亦その核型
も $K(n)=20=2V(H)+4J+12(4v+8j)+2m(h)$
で丁度基本種のそれの2倍を示す。併し本種の2
個の H に由来する異常凝縮塊の大きさには差が
認められ，一方は他方に比して常に大きく，且こ
の差は体止核では一層顕著であつた.(Fig. I, m)。

Pohlia nutans, P. patentissima. (Fig. I, n-q)

両種共雌雄異株，染色体数は $n=22$ であつた。
筆者はすでに P. nutans, P. longicollis の両種が
雌雄同株で $n=11$ を基本数とする二倍種である
こと，及び $K(n)=22=2V(H)+12J+6+2m(h)$
の核型式で示されることを報告した（矢野1953a）。

今回は P. patentissima と共に P. nutans につ
いても精しく再観察を行い，その結果前報告では
明らかになし得なかつた小型染色体の狭窄の位置
もほゞ決定することが出来た。両種の染色体組は
上記各種の場合と同様に大小二群の染色体に分け

られるので，改めて次の 核型式で示すことゝす
る。$K(n)=22=2V(H)+4J+14(4v+10j)+2 m$
(h)。

尚この2個の H 間には，両種共，P. Suzukii
の両 H 間に認められたと同様な異常凝縮性の差
が見られた。本核型を P. Suzukii のそれと比べ
ると常染色体に於て2個の微小な j が余分に加わ
つており，亦上記の高一倍種（$n=11$）のそれに比
べると丁度その2倍となつている。従つてこれ等
両種は高二倍種に相当する。

尚 P. nutans に於ては胞子母細胞の減数分裂
をも観察した。その結果第一中期に於て 22II が
明瞭に認められ，多価染色体の形成及び不等対の
存在は認められなかつた （Fig. I, q）。

II. *Bryum*

Bryum nagasakense, B. argenteum, B. cyclo-
phyllum, B. pseudo-alpinum, B. caespiticum
(Fig. II)

各種共雌雄異株，研究に用いられた配偶体の性
別は不詳であつた。これ等のうち B. caespitic-
um の染色体数は Él. & Ém. Marchal (1911),
Wettstein (1924), Wettsetin & Straub (1942)

Fig.　II
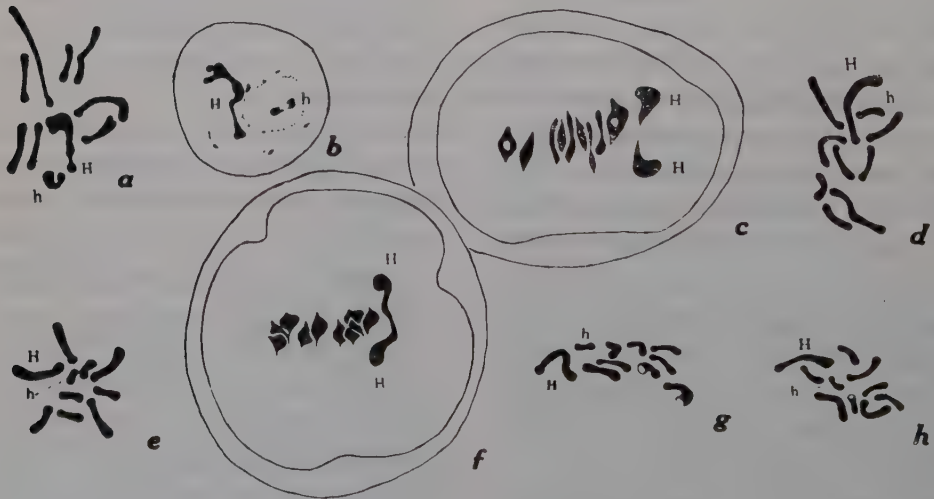
Fig. II. Chromosomes and heteropycnosis in five *Bryum*-species, $n=10$. a=c. B. *nagasakense*:
a, b. gametophyte; c. meiotic chromosomes at MI of SMC. d. B. *argenteum*, gametophyte.
e, f. B. *cyclophyllum*: e. gametophyte; f. meiotic chromosomes at MI of SMC.
g. B. *pseudo-alpinum*, gametophyte. h. B. *caespiticnm*, gametophyte.　×3000

により $n=10$, Griesinger (1937) により $n=10$ 及び $n=20$, 三宮 (1955) により 20 II と報告され, B $argenteum$ のそれは Ém. Marcha l(1920) により $n=10$, Jachimsky (1935) により $n=7$ と報告されている。筆者によつて研究された上記 5 種の染色体数はすべて $n=10$ であり, 亦各種の染色体組の形態も互に酷似している。即ち各染色体組は 4 個の大型染色体 (2V, 2J), 5 個の小型染色体 (ほゞ 2v, 3j) 及び微小な m より成る。そし

詳, $n=10$。各染色体の形態, 異質染色体 H, h の異常凝縮性等は $Bryum$ 属のそれ等とほゞ同様である。然し本種の染色体の大きさは $Bryum$ 属のそれ等に比し一般に幾分小さい。 $K(n)=10=$ V (H)+V+2J+5(2v+3j)+m(h).

IV. *Plagiobryum*

Plagiobryum japonicum. (Fig. III, c, d)

雄雌異株, 研究に用いられた配偶体の性別不詳。$n=10$。この染色体も大小の二群に分けられ

Fig. III
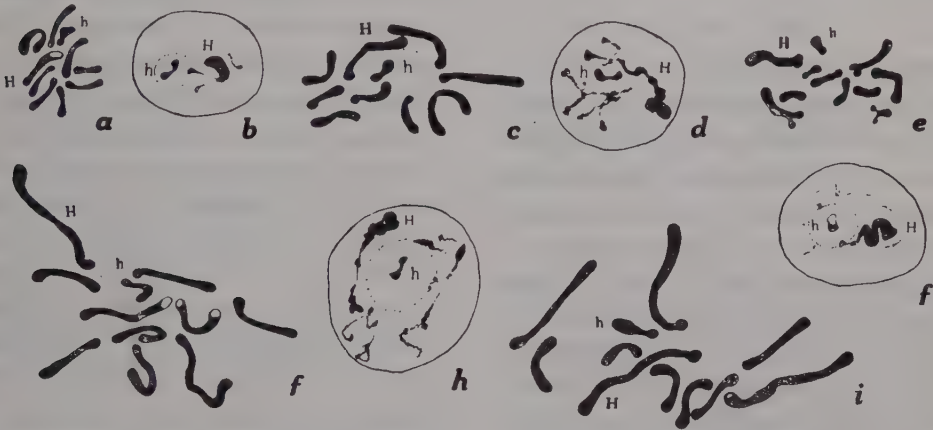
Fig. III. Gametoyhytic chromosomes and heteropycnosis of *Anomobryum japonicum* (a, b), ($n=10$) *Plagiobryum japonicum* (c, d), ($n=10$), *Brachymenium exile* (e, f) ($n=11$), *Rhodobryum roseum* (g, h) ($n=11$), and *R. giganteum* (i) ($n=11$). ×3000

て最大の V 及び m はそれぞれ H 及び h である。H は median の着糸点を有し左腕の端部に二次狭窄を有し, 右腕の大半が heteropycnotic である。m は全部が heteropycnotic であり, これは Nukleolinus-Chromosomen である。従つてこれ等各種の核型は共通に $K(n)=10=V(H)+V+2J+5(2v+3j)+m(h)$ で示される。

$B.$ $nagasakense$ 及び $B.$ $cyclophyllum$ の両種では胞子母細胞の減数分裂も観察された (Fig. II, c, f)。その結果両種共第一中期で 10II が明瞭に認められ, 最大の H–H 対合は屢々特異な形態を示し, 且これは後期で他より先んじて分離し始めるが両極に達するのはむしろ遅れることが多かった。

III *Anomobryum*

Anomobryum japonicum (Fig. III, a, b)

雌雄異株, 研究に用いられた配偶体の性別不

る――大型の 4 個 (2V, 2J), 小型の 5 個 (ほゞ 2v, 3j) 及び m。最大の V 及び m はそれぞれ $Bryum$ 属のそれ等と同様な H, h である。従つて核型は $Bryum$ 属と同様 $K(n)=10=V(H)+V+2J+5(2v+3j)+m(h)$ で示される。然し本種の染色体は $Bryum$ のそれ等に比し一般に幾分大きい。

V. *Brachymenium*

Brachymenium exile (Fig. III, e, f)

雌雄異株, 研究に用いられた配偶体の性別不詳. $n=11$。この染色体組は 4 個の大型染色体 (2V, 2J), 6 個の小型染色体 (ほゞ 3v, 3j) 及び微小な m より成る。小型の 3j のうち 2 個は殊に微小でほゞ m の大きさと同様である。最大の V 及び m はそれぞれ H 及び h である。

$K(n)=11=V(H)+V+2J+6(3v+3j)+m(h)$

VI. *Rhodobryum*

Rhodobryum roseum, R. giganteum. (Fig. III, g-i)

雌雄異株．研究に用いられた配偶体の性別不詳．*R. giganteum* の染色体数は下斗米，小山 (1932) により $n=11$ と報告され，亦筆者も既に上記両種で $n=11$ と報告している (矢野1952)．今回は更に個々の染色体の形態を観察して次の如き核型を定めた． $K(n)=11=V(H)+5V+4J+m$ (h)．　両種の染色体は他の蘚に比して著しく大きい．

考　察

Pohlia 属の10種で $n=10$, $n=11$, $n=20$. 及び $n=22$ の染色体数が算定された．これ等のうち $n=10$ の *P. scabridens* は本属の基本種であり，$n=11$ の 6 種 (*P. revolvens, P. columbica, P. cornea, P. Wahlenbergii, P. revoluta, P. acuminata*) は基本核型に比し1個の微小な染色体 (j) を過剰にもつている高一倍種，$n=20$ の *P. Suzukii* は基本核型の 2 倍を示す二倍種，$n=22$ の 2 種 (*P. nutans P. patentissima*) は高一倍種の核型の 2 倍を示す高二倍種であつた．そして $n=10$ 及び $n=11$ の各種は何れも雌雄異株であるが $n=20$, $n=22$ の各倍数種は雌雄同株であつた．この性分化の相違は，各一倍種 ($n=10$, $n=11$) の雌株及び雄株の H はそれぞれ性染色体 X 及び Y であり，各二倍種 ($n=20$, $n=22$) がもつている 2 個の H もそれぞれ一倍種の雌株及び雄株から由来した X 及び Y であると仮定すれば容易に理解される．Él. & Ém. Marchal (1907, '09, '11) は雌雄異株の蘚の sporophyte から人為的 apospory によつて生じた配偶体は雌雄同株であることを実証し，自然界でも同様な再生によつて倍数体蘚が生づる可能性があることを示した．一方 Müntzing (1933), Heitz (1942) 及び Lowry (1948, '54)

等も雌雄同株蘚は倍数性の所産であろうと主張し，亦筆者も倍数性の所産と推定される多くの雌雄同株蘚の例を報告している (矢野 1951, '53a, '53b, '54, '55a, '55b)．今回研究された *Pohlia* 雌の倍数性は更に此種の例を加えたものであり，且これは上記各先人の主張を支持するものである．尚 $n=20$, $n=22$ の各二倍種の 2 個の H 間には異常凝縮性の差が認められるので両者間には構造的な差があるものと推定される．従つて各一倍種 ($n=10$, $n=11$) の雌雄の H 間にもおそらく同様な差があるものと推察される．併しそれ等の雌株及び雄株を別々に培養することが困難であつた為，今回は此の比較は行われなかつた．而して此の点は将来明らかにし度いところである．

Bryum 属の染色体数は多くの研究者によつて (Él. & Ém. Marchal 1911, Ém. Marchal 1920*, Heitz 1928, Jachimsky 1932, Griesinger 1937*, 三宮 1955) その 4 種について報告されている (*B. capillare B. caespiticum, B. argenteum, B. Corrensii*)．それ等の染色体数は *B. argenteum* につき Jachimsky が $n=7$ と報告したものを除けばすべて $n=10$ 又は $n=20$ であつた．亦筆者によつて研究された 5 種 (*B. nagasakense, B. argenteum, B. cyclophyllum. B. pseudo-alpinum, B. caespiticum*) もすべて $n=10$ であつたので，本属の基本数はおそらく $n=10$ であろう．尚 *B. caespiticum* につき Griesinger (1937)* は $n=10$ 及び $n=20$ と報告しているが，最近三宮 (1955) も邦産の本種の減数分裂を観察し 20II を算定した．筆者の材料は $n=10$ であつたので，邦産の本種にも種内倍数性が存在することが明らかとなつた．

本研究の遂行につき御指導を賜つた辰野博士，並びに研究材料の同定をしていただいた野口博士及び越智春美氏に対し深く謝意を表する．

Résumé

Six genera twenty species of mosses belonging to Bryaceae were studied cytologically with special reference to their karyotypes and heterochromosomes. The results obtained are as follows:

Pohlia scabridens (Mitt.) Broth.　　　　$K(n)=10=V(H)+2J+6(2v+4j)+m(h)$

P. revolvens (Card.) Nog.　　　$K(n)=11=V(H)+2J+7(2v+5j)+m(h)$

P. revoluta (Card.) Ochi.　　　$K(n)=11=V(H)+2J+7(2v+5j)+m(h)$

P. columbica (Kindb.) Broth.　　$K(n)=11=V(H)+2J+7(2v+5j)+m(h)$

P. cornea (L.) Limpr.　　　$K(n)=11=V(H)+2J+7(2v+5j)+m(h)$

P. Wahlenbergii (Web. et Mohr.) Andrews.　$K(n)=11=V(H)+2J+7(2v+5j)+m(h)$

P. acuminata Hoppe et Hornsh.　$K(n)=11=V(H)+2J+7(2v+5j)+m(h)$

P. Suzukii Ochi.　　　$K(n)=20=2V(H)+4J+12(4v+8j)+2m(h)$

P. nutans Schreb.　　　$K(n)=22=2V(H)+4J+14(4v+10j)+2m(h)$

P. patentissima Ochi.　　$K(n)=22=2V(H)+4J+14(4v+10j)+2m(h)$

Bryum nagasakense Broth.　$K(n)=10=V(H)+V+2J+5(2v+3j)+m(h)$

B. argenteum L.　　　$K(n)=10=V(H)+V+2J+5(2v+3j)+m(h)$

B. cyclophyllum (Schwagr.) Br. eur.　$K(n)=10=V(H)+V+2J+5(2v+3j)+m(h)$

B. pseudo-alpinum Ren. et Card.　$K(n)=10=V(H)+V+2J+5(2v+3j)+m(h)$

B. caespiticum L.　　　$K(n)=10=V(H)+V+2J+5(2v+3j)+m(h)$

Anomobryum japonicum Broth.　$K(n)=10=V(H)+V+2J+5(2v+3j)+m(h)$

Plagiobryum japonicum Nog.　$K(n)=10=V(H)+V+2J+5(2v+3j)+m(h)$

Brachymenium exile (Doz. et Molk.)

v. d. B. et Lac.　　　$K(n)=11=V(H)+V+2J+6(3v+3j)+m(h)$

Rhodobryum roseum (Weis.) Limpr.　$K(n)=11=V(H)+5V+4J+m(h)$

R. giganteum (Hook.) Par.　　$K(n)=11=V(H)+5V+4J+m(h)$

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タバコ属植物の細胞遺伝学的研究　X*
N. Langsdorffii の減数分裂

竹　中　　要**

Yô TAKENAKA: Cytogenetic Studies in *Nicotiana* X.
Reduction Divisions in *N. Langsdorffii*.

本研究の目的は，(1) 種間交雑による育種的優良形質の発見，(2) 雑種の減数分裂異常を利用しての優良劣悪形質の分離，(3) タバコ属の核学的研究方法による系統進化の究明である。

材料と研究方法

本研究に用いた *N. Langsdorffii* は米国の Howard 氏から 日本専売公社に 寄贈されたものである。本種は *Alata* 節に属し染色体数 $2n=18$ である。かつて 外形的の 観察から 分類学者に よつて

る "なすりつけ法" に従つた。しかし時にはカルノー氏液にて葯を固定した後，塩酸で処理して醋酸オルセインによる "おしつぶし法" を併用した。

観　　察

花粉母細胞の減数分裂前期は通常の型に従つて進行する。移動期に入ると 2 価染色体が 7 組と 4 価染色体が 1 個と見られる。9 組の 2 価染色体を観察することもあるが，その数は前者に比し少ない。4 価染色体の中央に位する 2 価染色体は染色体中最大のものであり，両端に位置する 2 箇は小さ
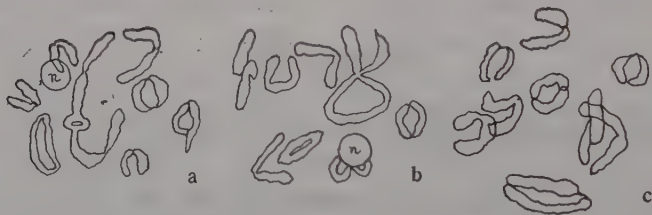
Fig. 1. *N. Langsdorffii*, PMC' s.

a, one type of early diaphase, $7_{II}+1_{IV}$.

b, other type of early diaphase, $7_{II}+1_{IV}$,
showing one heteromorphic pair.

c, diaphase, 9_{II}, showing one heteromorphic pair.

Rustica 節に入れられていたが，交配研究の結果，今日では上記の通り *Alata* 節に入ることになつた。花は *N. rustica* に似て小形で，やや鈎鐘状を呈し，花色は黄緑のものと褐紫のものとある。花粉が *Petunia violacea* のように菫碧色であるのが本種の著しい特徴である。

花粉母細胞の観察は多くは鉄醋酸カーミンによ

い。最大の染色体について大きい染色体が 1 対ある。他の 6 組の 2 価染色体と 4 価染色体の両端に位するものとはほぼ同大である。仁染色体は 1 対である。

第一中期には 9 組の 2 価染色体の核板もあるが，多数は 7 組の 2 価と 1 箇の 4 価染色体の核板を見る。稀れには 7 組の 2 価，1 箇の 3 価，1 箇
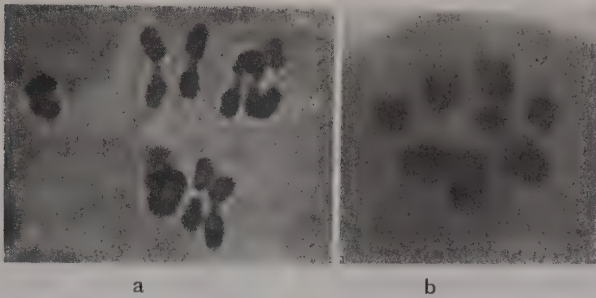
Microphoto. of MI in PMC's of *Nicotiana*
Langsdorffii. a, $7_{II}+1_{IV}$, b, $6_{II}+1_{VI}$.

Fig. 2.
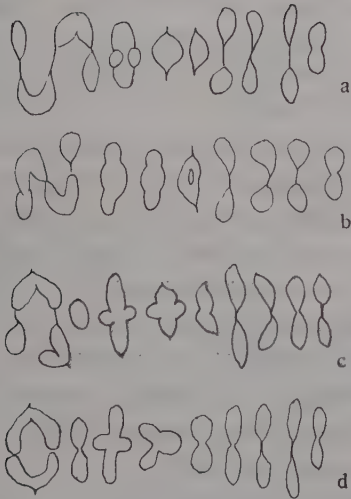
Fig. 2. *N. Langsdorffii*, MI, chromosome
figs. a, b, $7_{II}+1_{IV}$. c, $7_{II}+1_{III}+1_I$. d, 9_{II}

の1価染色体からなる像も見られる。9 組の2価
染色体の場合は1箇の大きな2価と，それにつづ
く大きさの1箇の2価染色体の外はほゞ同じ大き
さの2価染色体である。1 箇の4価染色体の構成
は中央の2箇は最大染色体であつて，両端の2箇
はなみの大きさである。4 価染色体の型は両端の
開いた n 字型である。稀れに3価染色体が見られ
るが，そのときは必ず別に1価染色体1箇が存在
する。この3価染色体の一端のものはなみの大き
さであり，中央と他端の染色体は最大のものであ
る。1 価染色体もまたなみの大きさのものであ
る。すなわち4価染色体の一端の染色体が接合し
なかつた場合に当る。4 価染色体にはごく稀れに
n 字型でなく v 字型のものが見られる。なお非常

に稀れに環状をなす1箇の6価染色体が観察され
た。今第一中期染色体の構成を示すと次のようで
ある。

第一中期染色体構成

染 色 体 構 成	9_{II}	$7_{II}+1_{IVII}$,	$7+1_{III}+1_I$	Total
頻 度	22	70	11	103

第一後期においては2価染色体は通常規則正し
く両極に分配されるが，稀れに染色体橋をつくる
ものがある。4 価染色体は両端の2箇は正しく

Fig. 3.
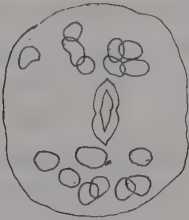
Fig. 3. *N. Langsdorffii*, AI, showing
a chromosome bridge builded from the
largest bivalent.

両極に行くが，中央の最大の2箇はときに分離が
おくれて染色体橋をつくるのみならず，この2つ
が2価染色体をつくると，しばしば染色体橋をつ
くる。これ以外で染色体橋を示すものが，この 4
価染色体の両端にあたるものが2価染色体をつく
つた場合に相当するかどうかは決定できなかつ
た。まれに染色体の断片化によつて 1～2 箇の小
染色体片が核板外に放出されることがある。

第二分裂中期では両核板の染色体は共に 9 が普通であるが，時には 9 と 8 であつて 1 染色体が遊離することがある。またごく稀れには 8 と 8 とであつて 2 箇の遊離染色体が見られることもあつた。第二分裂後期では稀れに 1 つの染色体橋が見られた。四分子期の母細胞は殆んど 4 分子をつくるが，稀れに 1 微細胞子を余分にもつものがある。

論　議

Avery (1938) は *Alata* 節の細胞遺伝学的研究から，その系統を論じたが，*N. Langsdorffii* の減数分裂の研究で第一中期に 9_{II} を見た。しかるに筆者はその花粉母細胞の約 70% に 1 箇の 4 価染色体をもつことを知つた。また 3 価染色体をもつものを同じ傾向をもつものとして算定すると，それは約 80% の多きに達する。このような植物は先ず第一に雑種性のものと考えられる。この 4 価染色体の中央の大きな 2 箇の染色体を P と P′ の記号で示し，両端の小さな 2 箇を Q と Q′ の記号で示すと，この 4 価染色体は Q P P′ Q′ である。P と P′ は分離に際して染色体橋を作るから相同部分に逆位をもつものと推定される。また Q と Q′ は 4 価または 3 価染色体の構成員とならないときは常に（全体の 20%）QQ′ のゲミニを構成する。つまり P と P′ は逆位になつた部分を含めて大きな部分に相同があり，Q は P との間に，Q′ は P′ との間に大きな相同部分をもつ外に Q と Q′ の間にも大きな相同部分がある。

Kostoff (1941—1943) は *N. Langsdorffii* の半数体を研究して，第一分裂の中期と後期のはじまりとに，しばしば 1 箇の，時には 2 箇の，ごく稀れに 3 箇の 2 価染色体を観察した。そしてその多くは端部に 1 箇のキアズマを持つており，少数は亜端部に 1 箇のキアズマを示したと報告した。そして端部キアズマのものは逆位を示さないが，亜端部キアズマのものは相同部が逆位であると推察した。

筆者の研究と併せて考えると，P と Q′ とが，或は P′ と Q とが半数体で接合を示したものと推察される。従つて上に述べた P と P′，Q′ と Q′，P と Q，P′ と Q′ 及び P と Q′（或は P′ と Q）の間にそれぞれ大きな部分相同が存在することになるが，このことは P，P′，Q，Q′ の 4 染色体が，逆位は含むが，殆んど相同の遺伝子構成であることを推察せしめる。

次に筆者は稀れに 6 価染色体を観察したが，Kostoff (1941—43) もまた半数体で移動期と第一中期に稀れに 3 価染色体を見た。これらの染色体の中に，上に述べた P (P′), Q (Q′) の染色体は恐らく含まれるものと思われる。従つて P (P′), Q(Q′), の外に X (X′) という相同部分をもつ染色体が考えられる。

Kostoff (1941—43) はまた半数体で，第一分裂中期の花粉母細胞で，接近した 2 箇の染色体を 1〜3 組観察した。それはあたかも二次対合を思わせるものであつた。彼はこの半数体の研究を主にして，タバコ属の基本染色体数を 6 と考えているが，筆者の研究も何等かの意味をもつて，タバコ属の基本染色体数が $n=9$ よりも低いものであることを推察せしめる。すなわち *Langsdorffii* は雑種起原であるかもしれないが，とにかく一種の Hyperploid と考えられる。

上のように 4 価染色体は Q P P′ Q′ の構成をなすから，その配偶子は Q P′ と P Q′ とである。そして Q P′ と P Q′ とを持つ受精卵のみ胚を完成するとするならば，2 種の卵核は 2 種の花粉からそれぞれに対応するものを選択せなければならぬ筈である。これが事実であるかどうかは今後研究さるべき事柄である。

Summary

The haploid chromosome number of *N. Langsdorffii*, is nine, as in *N. alata*, *N. Sanderae* and *N. bonariensis*. Avery (1938) reported that this plant shows nine bivalents at the first metaphase of the PMC's; however, I have most often observed the configuration $7_{II}+1_{IV}$, occasionally 9_{II}, and rarely $7_{II}+1_{III}+1_{I}$ at the diakinesis and also at the first metaphase. The quadrivalent is N-shaped at the first metaphase. The two middle elements are the largest of all the chromosomes and the two ter-

minal ones are about the same in size as the other chromosomes. At the first ana-
phase, the four elements of the quadrivalent are distributed equally between the
two poles but the two large middle elements showes frequently a chromosome
bridge.

At the meiosis of haploid *N. Langsdorffii*, Kostoff (1930, 1938, 1941) observed
one, two or three groups of two chromosomes in close proximity (secondary associa-
tion) during the first metaphase, and occasionally one, rarely two and more rarely
three bivalents during the first metaphase and early anaphase. Accordingly, he as-
sumed that the basic chromosome number of the ancestral plant of the genus *Nico-
tiana* might have been six. My observations also suggest that the basic chromosome
number of this genus is lower than 9.

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禾 本 科 牧 草 数 種 の 核 型 分 析

柴　　田　　寛　　三 *

Kanzō SHIBATA : Karyotype Analysis on Some Forage Grasses.

1956 年 1 月 26 日受付

筆者は *Lolium perenne* L. (Perenial rye gra-
ss), *L. multiflorum* Lam. (Italan rye grass),
L. temulentum L. (ドクムギ), *Arrhenatherum
elatius* (Linn.) Mert. et Koch. (Tall oat grass)
及び *Sorghum vulgare* L. var *sudanese* Piper.
(Sudan grass) の 3 属 5 植物について核型分析を
行つた。その結果をここに報告する。

本研究に使用した材料の中，ドクムギは東京農
業大学茂原分校構内野生の植物より採種した種子
を用い，他の 4 植物の種子は東京の種苗店より購
入した種子を用いた。それらの種子をシャーレー

にまき，発芽した根端細胞が用いられ，前処理及
び固定染色方法は Tjio & Levan (1950) の方法
を一部変更した方法が用いられた。即ち 22～24°
C において 0.002 mol. の 8—Oxy-quinoline
水溶液に 3 時間浸し，30 分間流水で水洗し，醋酸
アルコール (1:3) で固定した後，Warmke (193
5) の方法で解離を行い，45 %醋酸に数時間浸し
た後，Aceto-orceine でおしつぶした。核型の表
わし方は篠遠 (1943) によつた。

観　　察

1. Perenial rye grass. *Lolium perenne* L.

* 東京農業大学茂原分校 (Mobara Branch, Tokyo Agricultural University, Mobala, Chiba Pref.,
Japan.)

本種の染色体数 について Katayama (1929),
Kattermann (1930), Nakajima (1930), Moriya
& Kondo (1950) 及び Takizawa (1952) は $n=7$,
Evans (1926) 及び Nilsson (1933) は $2n=14$,
Peto (1934) は $n=7, 2n=14$ と報告している。

筆者も本種の染色体数を $2n=14$ と決定した。
これら14個の染色体はその形及び大きさから7対
に区別することが出来る。即ち最大の1対は
median 着糸点をもつている。次の 1 対は sub-
median に着糸点をもち,長腕にそれぞれ二次狭窄
を有し 第3対は median に着糸点をもつてい
る。これよりやゝ小さい第4対は submedian に
着糸点をもち, 長腕にそれぞれ二次狭窄を有し,
第5対は submedian に, 第6対及び最小の1対
はそれぞれ submedian に着糸点を も つ ている
(Fig.1)。核型は次の式で表わされる。

$$K(2n)=14=2A_1^m+2_{cs}A_2^{sm}+2B_1^m+$$
$$2_{cs}B_2^{sm}+2C_1^{sm}+2C_2^{st}+2C_3^{st}$$

2. Italian rye grass. *Lolium multiflorum* Lam.

本種の染色体について Jenkin (1924) 及び Av-
dulor (1928. 1931) は $2n=14$ と報告している。

筆者も本種の染色体数を $2n=14$ と決定した。
これら14個の染色体はその形及び大きさから7対
に区別することが出来る。即ち最大の1対は

submedian に着糸点をもつている。次の 1 対も
submedian に着糸点をもち, 長腕にそれぞれ二
次狭窄を有し, 第3対は median に, 第4対は
submedian に着糸点をもつている。それより僅
かに小さい第5対は submedian に着糸点をも
ち, 長腕にそれぞれ二次狭窄を有し, 第6対は
submedian に, 最小の1対は subterminal に着
糸点をもつている (Fig. 2)。核型は次の式で表わ
される。

$$K(2n)=14=2A_1^{sm}+2_{cs}A_2^{sm}+2B_1^m+$$
$$2B_2^{sm}+2_{cs}B_3^{sm}+2C_1^{sm}+2C_2^{st}$$

3. ドクムギ *Lolium temulentum* L.

本種の染色体について Jenkin (1924) は $2n=$
14 と報告している。

筆者も本種の染色体数を $2n=14$ と決定した。
これら14個の染色体はその形及び大きさから7対
に区別することが出来る。即ち最大の1対は
median に, 次の1対は submedian に, 第3対
は subterminal に, 第4対は median に, やゝ
小さい第5対は submedian に; 第6対は sub-
terminal に, 最小の1対は median に着糸点を
つている (Fig. 3)。核型は次の式で表わされる。

$$K(2n)=14=2A_1^m+2A_2^{sm}+2A_3^{sm}+2A_4^m$$
$$+2B_1^{sm}+2B_2^{st}+2C_1^m$$

Figs.　1—5
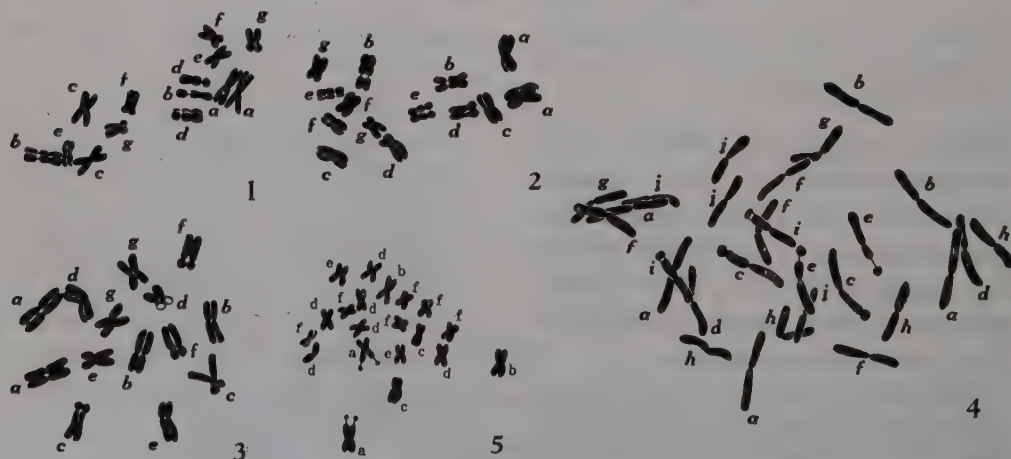
Figs. 1~5. Somatic nuclear plates in the root tip cells, treated in an aqueous solution of 0.002 mol/l 8-Oxyquinoline for 3 hours and stained with acetic-orcein. 1. *Lolium perenne*. 2. *L. multiflorum*. 3. *L. temulentum*. 4. *Arrhenatherum elatius*. 5. *Sorghum vulgare* var. *sudanese*. Ca. × 950

4. Tall oau grass. *Arrhenatherum elatius*
　　　(Linn.) Mert. et Koch.

本植物の染色体について Aase & Powers (1926), Nakajima (1931) 及び滝沢 (1952) は n=14, Kattermann (1930) 及び Avdulov (1931) は 2n=28 と報告してゐる。

筆者も本植物の染色体数を 2n=28 と決定した。これら28個の染色体はその形及び大きさから10種類に区別することが出来る。即ち最大の4個は submedian に, 次の1対はmedeianに着糸点をもつている。第3の1対は submedian に着糸点をもち, 短腕にそれぞれ二次狭窄を有する。第4の1対は median に着糸点をもつている。それより僅かに小さい第5の1対は submedian に着糸点をもち, 短腕に大型の附随体を有する。第6の4個は submedian に, 第7の1対は median に, 第8の4個は submedian に着糸点をもつている。又これより小さい第9の1対は median に着糸点をもち, 短腕にそれぞれ二次狭窄を有する。最小の4個は median に着糸点をもつている (Fig.4)。核型は次の式で表わされる。

$$K(2n)=28=4A_1^{sm}+2A_2^m+2^{cs}A_3^{sm}+2A_4^m$$
$$+2^t B_1^{sm}+4B_2^{sm}+2B_3^m+4B_4^{sm}+2^{cs}C_1^m+4C_2^m$$

5. Sudan grass. *Sorghum vulgare* L. var.
　　　sudanese Piper.

本植物の染色体数は筆者により 2n=20 と算定された。これら 20 個の染色体は前記4種の染色体より小さい。それらをその形及び大きさから6種類に区別される。即ち最大の1対は submedian に着糸点をもち, 長腕にそれぞれ小さい附随体を有する。次の1対は median に, 第3の1対は submedian に, 第4の6個, 第5の1対及び最小の6個は median に着糸点をもつている (Fig 5)。核型は次の式で表わされる。

$$K(2n)=20=2_t A_1^{sm}+2B_1^m+2B_2^{sm}+6B_3^m+2B_4^m+6C_1^m$$

考　察

Lolium 属の3種はいずれも 2n=14 であり, その外部形態もよく似ている。その中 *L. perenne* 及び *L. multiflorum* は有用な牧草であり, *L. temulentum* は禾本科野生植物中唯一の有毒植物である。Jenkin (1924, 1931, 1934) は *L. temulentum* と *L. perenne* 及び *L. multiflorum* とは Section を別にし, これに対し, *L. multiflorum* は *L. perenne* と別種にすべきではなく, *L. perenne* の一変種とし, *L. perenne* var. *multiflorum* と分類すべきであると論じた。筆者の核型分析の結果よりしても *L. perenne* と *L. multiflorum* との間にはその核型に於いてもこまかい点で若干の相異はあるが, これは別種とすべき程のものではなく, Jekin の考えの妥当性を支持するものである。これに対し, *L. temulentum* は上述の2植物とその核型に於いて大いに異つて居り, 類縁関係は相当遠いものと思われる。

Sudan grass (*Sorghum vulgare* var. *sudanese*) の核型は Huskins & Smith (1932) 及び森谷 (1936) が *Sorghum vulgare* で行つた染色体の形態的特徴についての記述とほゞ一致する。

Résumé

1) The chromosome numbers and morphologies were studied in the species of *Lolium*, one species of *Arrhenatherum* and one species of *Sorghum*.

2) The karyotype formulae were as follows:

　Lolium perenne　　$K(2n)=14=2A_1^m+2_{cs} A_2^{sm}+2B_1^m+2_{cs}B_2^{sm}+2C_1^{sm}+2C_2^{st}+1C_3^{st}$

　L. multiflorum　　$K2(n)=14=2A_1^m+2_{cs} A_2^{sm}+2B_1^m+2B_2^{sm}++2_{cs}B_3^{sm}+2C_1^{sm}+2C_2^{st}$

　L. temulentum　　$K(2n)=14=2A_1^m+2A_2^{sm}+2A_3^{st}+2A_4^m+2B_1^{sm}+2B_2^{st}+2C_1^m$

　Arrhenatherum elatius　　$K(2n)=28=4A_1^{sm}+2A_2^m+2^{cs}A_3^{sm}+2A_4^m+2^t B_1^{sm}+$
　　　　　　　　　　　　　　　$4B_2^{sm}+2B_3^m+4B_4^{sm}+2^{cs}C_1^m+4C_2^m$

　Sorghum vulgare var. *sudanese*　　$K(2n)=20=2_t A_1^{sm}+2B_1^m+2B_2^{sm}+6B_3^m+2B_4^m+6C_1^m$

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小倉　謙　教授　記念号

　本年秋に，**小倉謙教授記念号**を出版する計画については，すでに昨年 11月号に簡単に予告しましたが，今回小倉謙教授功績記念会からの本出版に対する寄附 10万円も決定しましたので，次の要項によつて**植物学雑誌 10 月号, 11月号**を合併して出版することに決定いたしました。広く会員諸賢の投稿をお願いします。

　　　　出版予定月日　　　　**昭和31年10月25日**

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　　　　論文の形式分量等

　　　　すべて本誌の一般投稿規定に準ずる。

　なお編集などのつごうもありますので，ご投稿予定の方は，予め編集幹事までご申込お願いします。

小倉　謙　教授　記念号

　昨秋広島の評議員会の議題になつた小倉謙教授記念号については，本年 2 月上旬の同教授功績記念会の実行委員会において本出版に対し 10 万円の寄附が決定されたので，2月 9日編集委員会に謀つて，植物学雑誌10-11月合併号として，広く会員から記念論文を募集して，約 150 頁の記念号を出版することに決定した。

植物科名に関する標準和名表 (II)

日本植物分類学会

　その後の経過及び一般凡例：　先に本誌第65巻200—203頁（1952年）に日本植物分類学会が選定した植物科名の標準和名表，第1部 顕花植物を発表したが，ここに第2〜6部として羊歯植物，蘚苔類，藻類，菌類（初めに別部門として発足した変形菌類を含む），地衣類に関するものを掲げる。1950年11月4日東京における会で，各部ごとに選出された委員がその後予め全会員に配布した「植物科名整理資料」によつて得た会員の意見を参考にして仮案を作り，更にこれを再び全会員に配布して意見を求めて最後的な案を作つた。これらの案の中，藻類以外は1952年10月12日東京の会において，藻類は1953年10月10日金沢の会において採択された。編纂の方針に関しては，先に決定した顕花植物の部の凡例を参考とすることが要望されたが，各部門で事情が異る点があることが1951年9月24日温海の会で承認されたので，必ずしも全く同一ではない。詳細に関してはそれぞれの部の凡例を参照されたい。

　部門によつては，科の学名の基になつている属の学名を仮名書きにして標準和名としたものを含んでいる。ラテン語又はラテン語化されたギリシャ語を仮名書きとする方式に関しては，日本植物分類学会会報第3号（1953年）に発表された「ラテン語の実際的なカナ文字化（案）」について金沢の会で討議され，更に「科名仮名文字化小委員会」を作つて検討することとし，委員には山田幸男，木村有香，佐藤正己，津山尚が選出された。その後委員相互の間で意見を交換した結果，1955年10月12日広島の会に上記の「カナ文字化（案）」が委員会の案として提出され，採択された。これによつて本会案の最後の発表形式が確定した。その際委員側から「phをパ行にし，yをイとせずに拗音のユとする（例：Polyblepharis をポリュブレパリスとする）ことは現在のラテン語学界における発音法の主流に沿うものであるから，将来はこの方法にならうべきものかも知れない。」との意見が補足的に紹介された（採択案では上記はポリブレファリスとなる）。なお温海の会において，人名など固有名詞にもとずく属名については，その本来の国なり地域なりの発音に従うことが要請されたので，これに従うことにした（例：Chantransiaceae はシャントランシア科）。

　以下の表中，科より上級の分類群の名や科の配列順は便宜に従つたもので，検討の対照となつたものではない。科名のラテン綴りもまた検討の対照となつたものではないが，使用された前例があり，且つその部門の委員の同意を得た場合には文法的に正しい綴りに従うようにした。1科について1和名を選び，新旧両仮名づかいで現わした。ラテン科名の右側に唯1つの和名の記してあるものは両者が同一の書き現わし方になつているもの，2つ並記してあるものは2番目のものが旧仮名づかいによるものである。

　終りに標準和名選定の計画に関して多年努力された日本植物分類学会の幹事や委員の方々，「植物科名整理資料」などの印刷について援助を得た文部省学術用語分科審議会及び文部省大学学術局学術課の方々に対して感謝する。（津山尚記）

第2部 羊歯植物

　凡例：1. 大体第1部 顕花植物に準じたが，科の配列は Engler-Diels: Syllabus 11版（1936）によつた。担当委員は田川基二（世話人），伊藤洋，百瀬静男である。

　2. *印を附したものは，すぐその上の *印のない科を更に分割して2科以上を認める場合を示す。

Equisetaceae	トクサ	Psilotaceae	マツバラン
Lycopodiaceae	ヒカゲノカズラ，ヒカゲノカヅラ	Isoetaceae	ミズニラ，ミヅニラ
Selaginellaceae	イワヒバ，イハヒバ	Ophioglossaceae	ハナヤスリ

* Botrychiaceae　ハナワラビ

* Helminthostachyaceae　ミヤコジマハナワラビ

Marattiaceae　リュウビンタイ(広義)

* Angiopteridaceae　リュウビンタイ(狭義)

Osmundaceae　ゼンマイ

Schizaeaceae　フサシダ

* Lygodiaceae　カニクサ

Gleicheniaceae　ウラジロ

Hymenophyllaceae　コケシノブ

Cyatheaceae　ヘゴ

* Dicksoniaceae　タカワラビ

Dipteridaceae　ヤブレガサウラボシ

Polypodiaceae　ウラボシ

* Aspidiaceae　オシダ, ヲシダ

* Aspleniaceae　チャセンシダ

* Blechnaceae　シシガシラ

* Cheilopleuriaceae　スジヒトツバ, スヂヒトツバ

* Davalliaceae　シノブ

* Plagiogyriaceae　キジノオシダ, キジノヲシダ

* Pteridaceae　ワラビ

* Vittariaceae　シシラン

Parkeriaceae (Ceratopteridaceae)　ミズワラビ, ミヅワラビ

Marsileaceae　デンジソウ, デンジサウ

Salviniaceae　サンショウモ, サンセウモ

* Azollaceae　アカウキクサ

第 3 部　蘚 苔 類

凡例:　1.　本邦産の科名のみを収録する予定であつたが，全般的な見透しをつかせることは研究上にも有益であるから重要なものは本邦に産しないものも掲げた。*印は本邦からはまだ知られていない科である。

2.　科の配列については苔類は Evans (1939) を参考として多少の 修正 を加え，蘚類は 主として Brotherus (1924, 1925) によつた。

3.　担当委員は堀川芳雄，服部新佐，野口彰であるが，世話人堀川芳雄が編集にあたつた。

苔　　類

Haplomitriaceae　コマチゴケ

Ptilidiaceae　テガタゴケ

Lepidoziaceae　ムチゴケ

Calypogeiaceae　ツキヌキゴケ

Cephaloziaceae　ヤバネゴケ

Cephaloziellaceae　コヤバネゴケ

Harpanthaceae　ウロコゴケ

Jungermanniaceae　ツボミゴケ

Marsupellaceae　ミゾゴケ

Plagiochilaceae　ハネゴケ

Scapaniaceae　ヒシャクゴケ

Schistochilaceae　オヤコゴケ

Madothecaceae　クラマゴケモドキ

*Goebeliellaceae　ゲーベリエラゴケ

Radulaceae　ケビラゴケ

Pleuroziaceae　ミズゴケモドキ, ミヅゴケモドキ

Frullaniaceae　ヤスデゴケ

Lejeuneaceae　クサリゴケ

Treubiaceae　トロイブゴケ

Fossombroniaceae　ウロコゼニゴケ

Blasiaceae　ウスバゼニゴケ

Pelliaceae　ミズゼニゴケ, ミヅゼニゴケ

Makinoaceae　マキノゴケ

Pallaviciniaceae　クモノスゴケ

Metzgeriaceae　フタマタゴケ

Riccardiaceae　スジゴケ, スヂゴケ

*Monocleaceae　ミミカキゴケ

Marchantiaceae　ゼニゴケ

Rebouliaceae　ジンガサゴケ, デンガサゴケ

*Sauteriaceae　アシブトゼニゴケ

Targioniaceae　チンピンゼニゴケ

Corsiniaceae　ゼニゴケモドキ

Ricciaceae　ウキゴケ

*Sphaerocarpaceae　ダンゴゴケ

Anthocerotaceae　ツノゴケ

蘚　　類

Sphagnaceae　ミズゴケ, ミヅゴケ

Andreaeaceae　クロゴケ

Fissidentaceae　ホウオウゴケ, ホウワウゴケ

Archidiaceae　ツチゴケ

Ditrichaceae　キンシゴケ

Bryoxiphiaceae　エビゴケ

*Seligeriaceae　コシッポゴケ

Dicranaceae　シッポゴケ

Pleurophascaceae　ツヤサワゴケ

Leucobryaceae　シラガゴケ

Calymperaceae　カタシロゴケ

Encalyptaceae　ヤリカツギ

Pottiaceae　センボンゴケ

Grimmiaceae　ギボウシゴケ，ギバウシゴケ

Disceliaceae　ヨレエゴケ

Ephemeraceae　カンムリゴケ

Funariaceae　ヒョウタンゴケ，ヘウタンゴケ

Oedipodiaceae　ロクロゴケ

Splachnaceae　マルダイゴケ

Schistostegaceae　ヒカリゴケ

Georgiaceae　ヨツバゴケ

Bryaceae　カサゴケ

Mniaceae　チョウチンゴケ，チャウチンゴケ

Rhizogoniaceae　ヒノキゴケ

*Hypnodendraceae　キダチゴケ

Aulacomniaceae　ヒモゴケ

Bartramiaceae　タマゴケ

*Spiridentaceae　キノボリスギゴケ

Erpodiaceae　ヒナノハイゴケ，ヒナノハヒゴケ

Ptychomitriaceae　チヂレゴケ

Orthotrichaceae　タチヒダゴケ

Rhacopilaceae　シバゴケ

Fontinalaceae　カワゴケ，カハゴケ

Climaciaceae　マンネンゴケ

Hedwigiaceae　ヒジキゴケ

Cryphaeaceae　ツルゴケ

Leucodontaceae　イタチゴケ

*Ptychomniaceae　スジイタチゴケ，スヂイタチゴケ

*Prionodontaceae　タイワントラノオゴケ，タイワントラノヲゴケ

Trachypodaceae　ムジナゴケ

Myuriaceae　ナワゴケ，ナハゴケ

Pterobryaceae　ヒムロゴケ

Meteoriaceae　ハイヒモゴケ，ハヒヒモゴケ

*Phyllogoniaceae　フナバゴケ

Neckeraceae　ヒラゴケ

Lembophyllaceae　トラノオゴケ，トラノヲゴケ

Hookeriaceae　アブラゴケ

Symphyodontaceae　ウニゴケ

Leucomiaceae　ホソハシゴケ

Hypopterygiaceae　クジャクゴケ

Theliaceae　ヒゲゴケ

Fabroniaceae　コゴメゴケ

Leskeaceae　ウスグロゴケ

Thuidiaceae　シノブゴケ

Amblystegiaceae　ヤナギゴケ

Brachytheciaceae　アオギヌゴケ，アヲギヌゴケ

Entodontaceae　ツヤゴケ

Plagiotheciaceae　サナダゴケ

Sematophyllaceae　ハシボソゴケ

Hypnaceae　ハイゴケ，ハヒゴケ

Rhytidiaceae　フサゴケ

Hylocomiaceae　ヒョクゴケ

Buxbaumiaceae　キセルゴケ

Diphysciaceae　イクビゴケ

Polytrichaceae　スギゴケ

第 4 部　藻　　類

　凡例：1. 各分類群の配列の順序は便宜上主として Fritsch: The structure and reproduction of the algae, I-II 並に Smith: Manual of phycology によつた。

　2. 問題が多く且つ余り我国の小，中，高等学校の教科書等に関係のない科は省いたものが尠くない。

　3. 藻類部門の担当委員は 山田幸男（世話人），稲垣貫一，中村義輝，米田勇一，広瀬弘幸，瀬川宗吉，田中剛，岡田喜一　である。

緑 藻 類

Chlamydomonadaceae　クラミドモナス

Sphaerellaceae　スファエレラ

Volvocaceae　オオヒゲマワリ，オホヒゲマハリ

Polyblepharidaceae　ポリブレファリス

Phacotaceae　ファコツス

Tetrasporaceae　ヨツメモ

Palmellaceae　パルメラ

Chlorodendraceae　クロロデンドロン

Chlorococcaceae　クロロコックム

Chlorellaceae　クロレラ
Oocystaceae　オーキスチス
Selenastraceae　セレナストルム
Hydrodictyaceae　アミミドロ
Coelastraceae　コエラストルム
Ulotrichaceae　ヒビミドロ
Microsporaceae　ミクロスポラ
Cylindrocapsaceae　キリンドロカプサ
Sphaeropleaceae　ヨコワミドロ
Ulvaceae　アオサ, アヲサ
Monostromataceae　ヒトエグサ, ヒトヘグサ
Prasiolaceae　カワノリ, カハノリ
Cladophoraceae　シオグサ, シホグサ
Chaetophoraceae　カエトフォラ
Trentepohliaceae　スミレモ
Coleochaetaceae　コレオカエテ
Pleurococcaceae　プレウロコックス
Oedogoniaceae　サヤミドロ
Mesotaeniaceae　メソタエニウム
Zygnemataceae　ホシミドロ
Mougeotiaceae　ヒザオリ, ヒザヲリ
Gonatozygaceae　ゴナトジゴン
Desmidiaceae　チリモ
Protosiphonaceae　プロトシフォン
Derbesiaceae　ツエノイト
Caulerpaceae　イワヅタ, イハヅタ
Bryopsidaceae　ハネモ
Dasycladaceae　カサノリ
Codiaceae　ミル
Valoniaceae　バロニア
Siphonocladiaceae　マガタマモ
Boodleaceae　アオモグサ, アヲモグサ
Anadyomenaceae　ウキオリソウ, ウキオリサウ
Phyllosiphonaceae　フィロシフォン
Vaucheriaceae　フシナシミドロ
Characeae　シャジクモ, シャヂクモ

不 等 毛 類

Tribonemataceae　トリボネマ
Botrydiaceae　フウセンモ

ヒカリモ類

Chromulinaceae　ヒカリモ
Hydruraceae　ミズオ, ミヅヲ

珪 藻 類

Discaceae　クモノスケイソウ, クモノスケイサウ
Soleniaceae　ツツガタケイソウ, ツツガタケイサウ
Biddulphiaceae　イトマキケイソウ, イトマキケイサウ
Rutilariaceae　ルチラリア
Fragilariaceae　オビケイソウ, オビケイサウ
Eunotiaceae　エウノチア
Achnanthaceae　アクナンテス
Naviculaceae　ハネケイソウ, ハネケイサウ
Epithemiaceae　エピテミア
Nitzschiaceae　ニッチア

渦 鞭 藻 類

Prorocentraceae　プロロケントルム
Dinophysiaceae　ジノフィシス
Gymnodiniaceae　ギムノジニウム
Noctilucaceae　ヤコウチュウ, ヤクワウチュウ
Peridiniaceae　ペリジニウム
Ceratiaceae　ツノモ

ミドリムシ類

Euglenaceae　ミドリムシ

褐 藻 類

Ectocarpaceae　シオミドロ, シホミドロ
Sphacelariaceae　クロガシラ
Cutleriaceae　ムチモ
Tilopteridaceae　チロプテリス
Dictyotaceae　アミジグサ, アミヂグサ
Myrionemataceae　ミリオネマ
Elachistaceae　ナミマクラ
Leathesiaceae　ネバリモ
Ralfsiaceae　イソガワラ, イソガハラ
Heterochordariaceae　マツモ
Chordariaceae　ナガマツモ
Myriocladiaceae　クロモ
Acrothrichaceae　ニセモズク, ニセモヅク
Stilophoraceae　ヒモマクラ
Spermatochnaceae　モズク, モヅク
Sporochnaceae　ケヤリモ
Desmarestiaceae　ウルシグサ
Punctariaceae　ハバモドキ

Asperococcaceae　コモンブクロ

Striariaceae　ヨコジマノリ

Scytosiphonaceae　カヤモノリ

Coilodesmaceae　エゾブクロ

Chnoosporaceae　ムラチドリ

Ishigeaceae　イシゲ

Dictyosiphonaceae　ウイキョウモ, ウキキャウモ

Chordaceae　ツルモ

Laminariaceae　コンブ

Lessoniaceae　レッソニア

Alariaceae　チガイソ

Fucaceae　ヒバマタ

Cystoseiraceae　ジョロモク

Sargassaceae　ホンダワラ, ホンダハラ

紅　藻　類

Porphyridiaceae　チノリモ

Goniotrichaceae　ベニミドロ

Phragmonemataceae　フラグモネマ

Bangiaceae　ウシケノリ

Erythropeltidaceae　イソハナビ

Compsopogonaceae　オオイシソウ, オホイシサウ

Chantransiaceae　シャントランシア

Batrachospermaceae　カワモズク, カハモヅク

Helminthocladiaceae　ベニモズク, ベニモヅク

Thoreaceae　チスジノリ, チスデノリ

Bonnemaisoniaceae　カギケノリ

Chaetangiaceae　ガラガラ

Gelidiaceae　テングサ

Dumontiaceae　リュウモンソウ, リュウモンサウ

Rhizophyllidaceae　ナミノハナ

Squamariaceae　イワノカワ, イハノカハ

Corallinaceae　サンゴモ

Grateloupiaceae　ムカデノリ

Gloiosiphoniaceae　イトフノリ

Endocladiaceae　フノリ

Tichocarpaceae　カレキグサ

Callymeniaceae　ツカサノリ

Calosiphoniaceae　ヌメリグサ

Nemastomaceae　ヒカゲノイト

Sebdeniaceae　オカムラグサ

Furcellariaceae　スズカケベニ

Solieriaceae　ミリン

Rhabdoniaceae　イソモッカ, イソモククワ

Rhodophyllidaceae　ロドフィリス

Hypneaceae　イバラノリ

Plocamiaceae　ユカリ

Sphaerococcaceae　キジノオ, キジノヲ

Sarcodiaceae　アツバノリ

Gracilariaceae　オゴノリ

Phyllophoraceae　オキツノリ

Gigartinaceae　スギノリ

Rhodymeniaceae　ダルス

Champiaceae　ワツナギソウ, ワツナギサウ

Ceramiaceae　イギス

Delesseriaceae　コノハノリ

Dasyaceae　ダジア

Rhodomelaceae　フジマツモ, フヂマツモ

藍　藻　類

Chroococcaceae　クロオコックス

Cyanidiaceae　イデユコゴメ

Dermocarpaceae　デルモカルパ

Chamaesiphonaceae　カマエシフォン

Pleurocapsaceae　プレウロカプサ

Oscillatoriaceae　ユレモ

Nostocaceae　ネンジュモ

Rivulariaceae　ヒゲモ

Scytonemataceae　スキトネマ

Brachytrichiaceae　アイミドリ, アヰミドリ

Capsosiraceae　カプソシラ

Stigonemataceae　スチゴネマ

第 5 部 菌　　類

凡例: 1. 菌類科名の選定にあたつては, 小林義雄が世話人となり, 菌類談話会で原案をつくり, 広く日本植物分類学会会員の意見を容れて改訂し, 更に討議立案して, これが 日本植物分類学会で 採択されたものである。菌類談話会内での原案分担は 藻菌類 (印東弘玄), 子嚢菌類 (小林義雄), 銹菌類 (平塚直秀), 担子菌類 (銹菌類以外, 今関六也, 小林義雄),;不完全菌類 (今関六也) である。

2. 菌類分類のシステムは現在大いなる 過渡期にあるので, ここでは昔から広く用いられているもの

したがつた。

　　3. 従来「○○病菌科」と云う名が少なからずあるが，その「病」は出来る限り除いて和名を設定した。

　　4. 植物教科書に載つているものの外に，植物病理学関係の重要なものは含めてある。

　　5. 変形菌部門は江本義数が担当委員となり，編纂は別部門として発足したものであるが，便宜上第 5 部の一部分として発表する。この部門に関してはすべての科を挙げた。

変 形 菌 類

Amaurochaetaceae　アマウロカエテ

Arcyriaceae　アルキリア

Ceratiomyxaceae　ケラチオミクサ

Collodermaceae　コロデルマ

Didymiaceae　ジジミウム

Heterodermaceae　ヘテロデルマ

Liceaceae　リケア

Lycogalaceae　リコガラ

Margaritaceae　マルガリタ

Physaraceae　フィサルム

Reticulariaceae　レチクラリア

Stemonitaceae　ステモニチス

Trichiaceae　トリキア

Tubulinaceae　ツブリナ

藻 菌 類

Plasmodiophoraceae　プラスモジオフォラ

Olpidiaceae　ツボカビ

Synchytriaceae　サビツボカビ

Monoblepharidaceae　モノブレファリス

Saprolegniaceae　ミズカビ，ミヅカビ

Pythiaceae　ピチウム

Peronosporaceae　ツユカビ

Albuginaceae　シロサビキン

Mucoraceae　ケカビ

Pilobolaceae　ミズタマカビ，ミヅタマカビ

Entomophthoraceae　ハエカビ，ハヘカビ

子 嚢 菌 類

Saccharomycetaceae　サッカロミケス

Taphrinaceae　タフリナ

Aspergillaceae　コウジカビ，カウヂカビ

Monascaceae　ベニコウジカビ，ベニカウヂカビ

Erysiphaceae　ウドンコカビ

Hypocreaceae　ニクザキン

Clavicipitaceae　バッカクキン，バクカクキン

Sphaeriaceae　スファエリア

Xylariaceae　マメザヤタケ

Dermateaceae　ヘリタケ

Helotiaceae　ヘロチウム

Sclerotiniaceae　キンカクキン

Bulgariaceae　ゴムタケ

Geoglossaceae　テングノメシガイ

Pyronemataceae　ピロネマ

Pezizaceae　チャワンタケ

Helvellaceae　ノボリリョウタケ

Eutuberaceae　カイキン

Laboulbeniaceae　ラブルベニア

担 子 菌 類

Melampsoraceae　メランプソラサビキン

Pucciniaceae　サビキン

Ustilaginaceae　クロボキン

Tilletiaceae　ナマグサクロボキン

Dacrymycetaceae　ベニキクラゲ

Tremellaceae　シロキクラゲ

Auriculariaceae　キクラゲ

Septobasidiaceae　モンパビョウキン，モンパビャウキン

Exobasidiaceae　モチビョウキン，モチビャウキン

Hypochnaceae　クズワタタケ，クヅワタタケ

Thelephoraceae　イボタケ

Clavariaceae　ホウキタケ，ハウキタケ

Hydnaceae　ハリタケ

Polyporaceae　サルノコシカケ

Agaricaceae　マツタケ

Rhizopogonaceae　ショウロ

Phallaceae　スッポンタケ

Clathraceae　カゴタケ

Lycoperdaceae　ホコリタケ

Geastraceae　ヒメツチグリ

Sclerodermataceae　ニセショウロ

Astraeaceae　ツチグリ

Nidulariaceae　チャダイゴケ

不完全菌類

Melanconiaceae　メランコニア
Moniliaceae　モニリア

Dematiaceae　デマチウム
Stilbellaceae　タモタケ
Tuberculariaceae　ツベルクラリア

第 6 部　地 衣 類

凡例：1.　全世界に産する科を網羅した。

2.　科の配列は大体に於て Zahlbruckner: Catalogus Lichenum Universalis によつた。

3.　この表は始め朝比奈泰彦，佐藤正己の両委員が作つた原案に対する会員の意見を整理検討して，世話人佐藤委員がまとめ，更に日本植物分類学会の採択を得たものである。

嚢子地衣類

Moriolaceae　モリオラ
Epigloeaceae　エピグロエア
Verrucariaceae　アナイボゴケ
Dermatocarpaceae　カワイワタケ，カハイハタケ
Pyrenothamniaceae　ピレノタムニア
Pyrenulaceae　サネゴケ
Phyllopyreniaceae　フィロピレニア
Trypetheliaceae　チクビゴケ
Paratheliaceae　パラテリウム
Astrotheliaceae　ホシミゴケ
Strigulaceae　アオバゴケ，アヲバゴケ
Pyrenidiaceae　アオウロコゴケ，アヲウロコゴケ
Xanthopyreniaceae　クサントピレニア
Pyrenotrichaceae　ピレノトリックス
Mastodiaceae　マストジア
Mycoporaceae　ミコポラ
Thamnoliaceae　ムシゴケ
Caliciaceae　ピンゴケ
Cypheliaceae　ヒョウモンゴケ，ヘウモンゴケ
Sphaerophoraceae　サンゴゴケ
Arthoniaceae　ホシゴケ
Graphidaceae　モジゴケ
Xylographidaceae　モクハンゴケ
Chiodectonaceae　ゴフンゴケ
Dirinaceae　ヘリブトゴケ
Roccellaceae　リトマスゴケ
Lecanactidaceae　イワボシゴケ，イハボシゴケ
Byssolomataceae　ビッソロマ
Chrysothrichaceae　ワタゴケ
Thelotremataceae　チブサゴケ

Diploschistaceae　キッコウゴケ，キッカウゴケ
Ectolechiaceae　エクトレキア
Gyalectaceae　サラゴケ
Coenogoniaceae　スミレモモドキ
Ephebaceae　ケゴケ
Pyrenopsidaceae　モツレノリ
Lichinaceae　リキナ
Collemataceae　イワノリ，イハノリ
Heppiaceae　ヘップゴケ
Pannariaceae　ハナビラゴケ
Stictaceae　ヨロイゴケ，ヨロヒゴケ
Peltigeraceae　ツメゴケ
Lecideaceae　ヘリトリゴケ
Phyllopsoraceae　フィロプソラ
Baeomycetaceae　センニンゴケ
Cladoniaceae　ハナゴケ
Stereocaulaceae　キゴケ
Umbilicariaceae　イワタケ，イハタケ
Acarosporaceae　ホウネンゴケ
Pertusariaceae　トリハダゴケ
Lecanoraceae　チャシブゴケ
Anziaceae　アンチゴケ
Parmeliaceae　ウメノキゴケ
Usneaceae　サルオガセ，サルヲガセ
Caloplacaceae　ダイダイゴケ
Teloschistaceae　テロスキステス
Buelliaceae　スミイボゴケ
Physciaceae　ムカデゴケ

担子地衣類

Coraceae　ケットゴケ

註：第1部 顕花植物の中の Amaranthaceae, Annonaceae, Podostemaceae, Simaroubaceae, Haloragaceae, Boraginaceae の綴りが間違つてはいないかとの質問を受けるが，これらは International Code of Botanical Nomenclature (1952年) の Nomina Familiarum Conservanda に従つたものである。

植物科名に関する標準和名表の正誤

(本誌 65 No. 769～770)

頁	行	誤	正
201	左 12	Taxodiaceae ヌマスギ	Taxodiaceae（広義）スギ
			*Taxodiaceae（狭義）ヌマスギ
201	左 15～16	クワウエウザン	クワウエフザン
201	左 17	Sciadopitidaceae	Sciadopityaceae
202	左下カラ 2	モウセンゴケ	モウセンゴケ，マウセンゴケ
203	右下カラ 11	ハエドクサウ	ハヘドクサウ

本会記事

支 部 通 信

関 東 支 部

5 月例会（5 月 8 日，於林業試験場）　ツツジを観る会.

6 月例会（6 月 18 日，於東大理，植物学教室）永井威三郎，ユーゴスラビヤ国見聞談.　武田久吉，高山植物について.　田辺和雄，高山植物のスライド映写.

9 月例会（9 月 17 日，同上）　西田誠，ミヤコジマハナワラビの担葉体における維管束の走行.　山崎義人，禾本科植物における胚の移植と接木.

11 月例会（11 月 19 日，同上）　草野俊助，三宅驥一，日本植物学会初期の状況について.

31 年 1 月例会（1 月 21 日，同上）　鳥山英雄, オジギソウ葉枕の細胞生理学的研究. 吉田治，ヒトリシズカの胚発生. 福島博, *Hydrurus foetidus* の生活誌と氷雪藻 *Chromulina Smithii*.

2 月例会（2 月 18 日，同上）　鈴木泰，伊豆諸島の植物分布について. 小野記彦，伊豆半島のアゼトウナの自然雑種集団. 木村陽二郎，パリー国立博物館の事など.

役 員 の 移 動

幹事長門司正三氏がパキスタンへ 1 年間ユネスコ技術員として出張するので，3 月から幹事長として原寛氏が会長から指命された。

関東支部選出評議員原寛氏が幹事長になったので，次点の伊藤洋氏が評議員になった。

α-Glucosidases of *Candida tropicalis* var. *japonica*

by Teruo Sawai*

沢井輝男: *Candida tropicalis* var. *japonica* の α–グルコシダーゼ

Candida tropicalis var. *japonica* is a strain of the wild yeast, which was isolated in 1942 by Hoshino from a soil wet with sulfite waste liquor. At that time Yamaguchi (1) named this yeast *Mycotorula japonica* var. *K. H.*, but recently Kobayashi (2) proposed to rename it as mentioned according to Lodder and Kreger-Van Rij's ne nomenclature of the yeast (3). As this yeast possesses strong ability to util xylose, several studies have so far been made with regard to the yeast manufac using sulfite waste liquor (4, 5, 6). As to the enzyme of this yeast a stud amylase was made by Yamaguchi (7), that of xylokinase by Sawai (8), and another strain of *C. tropicalis* that of trehalase by Lukes and Phaff (9 present author attempted to investigate other carbohydrases of this yeas paper deals with the descriptions about the kinds and some properties of sidases which act upon four substrates: sucrose, maltose, methyl–α–glucos trehalose.

Methods

The yeast was grown in a semisynthetic medium. Basal medium contain liter (distilled water + tap water, 9 : 1) 6 gr. of $(NH_4)_2SO_4$, 2 gr. of KH_2PO gr. of $MgSO_4 \cdot 7H_2O$, 0.3 gr. of $CaCl_2 \cdot 2H_2O$ and 30 ml. of yeast extract. 100 ml ml. of this solution were given to 500 ml. flasks, sterilized, and added with source (sucrose, methyl–α–glucoside, dextrin, starch, mannose or mannitol) in th of aqueous solution which had been separately sterilized so as to make 4 to cent in the final medium. The flasks, after inoculation with the yeast which been precultured in the same medium containing glucose, were shaken for two in an incubator at 30°. The cells were harvested by centrifugation and wa twice with distilled water.

Three different sorts of enzyme preparation were used for the experiments, wl were prepared as follows. (a) The washed cells were treated with chilled acetc and dried *in vacuo* over P_2O_5. The dried cells thus obtained were suspended distilled water — unless otherwise stated, 0.30 gr. in 60 ml. water — and k overnight in a refrigerator. This was designated as "dry cell suspension".

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* Biological Institute, Aichi Gakugei University, Nagoya, Japan 愛知学芸大学生物学教室

preliminary experiments showed that the activity of each α-glucosidase of this yeast varied remarkably depending on the carbon source of the culture media, various samples of the dry cell suspensions which had been obtained with different carbon sources were used. (b) The washed cell paste was mixed about 5 per cent its weight of toluene and 0.3 to 0.5 per cent of $(NH_4)_2HPO_4$, autolyzed overnight at 25° or 30°, and after dilution with distilled water, kept overnight in a refrigerator. The autolyzate was centrifuged and the supernatant liquid, having been cleared by filtration, was dialyzed overnight in a cellophane bag against diluted buffer of pH 4.0 or 6.6 in the cold. This was designated as " autolyzate ". (c) The autolyzate was treated with alumina gel B at pH 5.5 to 5.8 and the enzyme was eluted with 1 per cent solution of $(NH_4)_2HPO_4$. This eluate designated as " B-eluate ". For pH adjustment of these enzyme solutions 0.5 N NH_4OH or acetic acid was used. All these enzyme preparations contained no fermentation system for glucose.

Methyl-α-glucoside was synthesized in our laboratory. Methyl-β-h-fructoside prepared with the aid of the transfructosidase activity of yeast invertase (10). substrates — sucrose, maltose, raffinose and trehalose — were the commercial cts of the reagent grade.

e reaction mixture consisted of 1 ml. of substrate solution (final concentration, , 1 ml. of HCl—citrate or phosphate–citrate buffer solution and 2 ml. of solution. The reaction vessels were incubated at 30° with occasional shaking. uitable lapse of time the enzyme action was stopped by the addition of 4 ml. nl. of 2 per cent sodium carbonate solution and the increase in the reducing was determined by the Somogyi's improved method (11). The percentage of drolysis was obtained from the graphs constructed beforehand with each sugar. nzyme activity was expressed in terms of the velocity constant (k) of the rder reaction, calculated with minutes as the time unit.

Results

Neutral α-glucosidase group. The dry cell suspension obtained from the es grown with sucrose as carbon source hydrolyzed sucrose, maltose and methyl– coside with the optimum pH of 6.1 to 6.6 (Fig. 1). This figure shows that v pH 4.0 this dry cell suspension acted upon none of these substrates. The mes containd in this suspension were found to be relatively labile, their activity g nearly lost by keeping the suspension overnight at pH 4.0 and at 30°. For purpose of determining whether or not the enzyme which hydrolyzed sucrose timally at pH 6.6 was β-h-fructosidase, the behavior of this enzyme toward ffinose and methyl-β-h-fructoside together with sucrose was examined using a eluate of high activity obtained by alumina gel adsorption. The results are presented in Fig. 2, which shows that raffinose and β-h-fructoside remained quite ffected even after sucrose was completely cleaved, Hence it appears likely that enzyme hydrolyzing sucrose of this yeast may not be a β-h-fructosidase but an
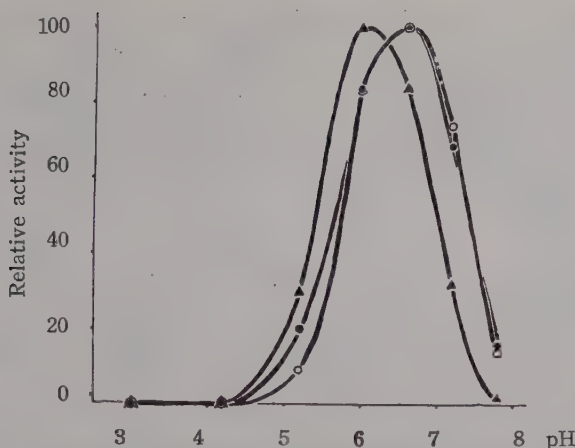
Fig. 1. Activity-pH-curves of the dry cell suspension obtained from the cells grown in a sucrose medium for the hydrolysis of sucrose (○), maltose (●) and methyl-α-glucoside (▲). Period of incubation: 180 min.
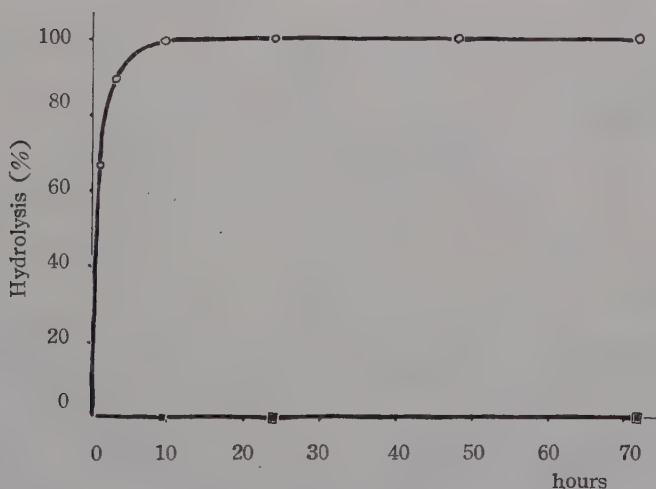
Fig. 2. Time-course of the hydrolysis of sucrose (○), raffinose (■) and methyl-β-h-fructoside (□) at pH 6.5 by the B-eluate obtained from the cells grown in a sucrose medium.

α-glucosidase.

In order to make clear whether one and the same enzyme is responsible for the hydrolysis of sucrose, maltose and methyl-α-glucoside or several specific enzymes are concerned, activity ratios toward these substrates were examined using a dry cell suspension and two kinds of autolyzates. The results are summarized in Table 1, which shows that the ratios of activity toward three substrates fluctuate quite irregularly. This fact might suggest that at least three kinds of the α-glucosidases would be involved in the hydrolysis of these substrates, but it is obscure whether or not each enzyme is responsible respectively for the hydrolysis of a single substrate. Accordingly they were referred to collectively as neutral α-glucosidase group.

Table 1. Activity of the neutral α-glucosidase group for the hydrolysis of sucrose, maltose and methyl-α-glucoside. As enzyme a dry cell suspension and two autolyzates were used which were obtained from the cells grown in a sucrose medium. The autolyzates were fractionated or partially inactivated as indicated. Reaction was carried out at pH 6.6 with sucrose and maltose and at pH 6.1 with methyl-α-gludoside.

Exp. No.	Reaction period, min. Substrate / Enzyme preparation		Velocity constant, $10^4 \cdot k$			Activity ratio *		
			Sucrose	Maltose	Me-α-gl.	Sucrose	Maltose	Me-α-gl.
1	Dry cell suspension	180	2.41	1.52	2.04	100	63	85
2	Autolyzate untreated (control)	120	20.34	10.03	11.89	100	49	58
	Autolyzate acidified to pH 4.0 and warmed at 30° for 40 min.	210	6.68	4.88	0.50	100	73	7
	Aqueous solution of the precipitate obtained by acetone treatment **	210	8.28	5.35	0.63	100	65	8
3	Autolyzate untreated (control)	1080	3.49	3.99	0.59	100	114	14
	Autolyzate heated at 50° for 7 min.	1080	0.58	2.32	0.23	100	400	40
	Aqueous solution of the precipitate obtained by tannin treatment	1080	0.65	0.58	0.20	100	89	31

* Activity for sucrose was taken as 100.

** Acetone treatment required 3.5 hours at room temperature.

(2) Acid α-glucosidase. With any other carbon source than sucrose such as methyl-α-glucoside, maltose, dextrin, starch or mannitol, this *Candida* yeast produced in addition to the neutral α-glucosidase group other enzymes which hydrolyzed maltose and methyl-α-glucoside optimally in more acid range. Fig. 3 shows the activity-pH-curves of the autolyzate obtained from the cultures in a dextrin medium for the hydrolysis of maltose and methyl-α-glucoside. The neutral α-glucosidases in the autolyzate had been destroyed by keeping it overnight at pH 4.0 and at 30°. It can be seen in Fig. 3 that for both substrates the optimum pH lies at about 4.0, where the neutral α-glucosidases are inactive. These enzymes were found to be stable enough to retain their entire activity even if the cell suspension was kept overnight at pH 3.5 and at 30° in contrast with the neutral α-glucosidase group, the activity of which was lost under these conditions (Table 2). The activity of
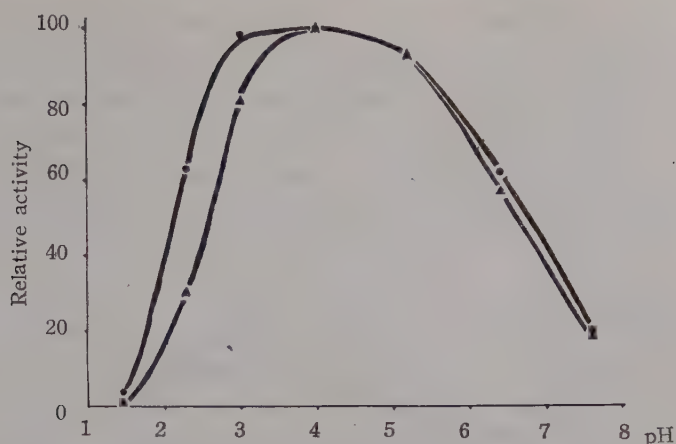
Fig. 3. Activity-pH-curves of the autolyzate obtained from the cells grown in a dextrin medium for the hydrolysis of maltose (●) and methyl-α-glucoside (▲). The autolyzate was freed from neutral α-glucosidases. Period of incubation: 180 min. with maltose and 1200 min. with methyl-α-glucoside.

Table 2. Stability in the acid medium of the α-glucosidase activity of the dry cell suspension obtaind from the cells grown in a dextrin medium.   The activity for maltose and methyl-α-glucoside was measured at pH 4.0.   Period of incubation: 240 min. with maltose and 1410 min. with methyl-α-glucoside.

Substrate / Dry cell suspension	Velocity constant, $10^4 \cdot k$		Relative activity *	
	Maltose	Me-α-gl.	Maltose	Me-α-gl.
Control	3.16	0.14	100	100
Kept overnight at pH 3.5 and at 30°	3.31	0.15	104.7	107.1

*   Activity of the control suspension was taken as 100.

these acid active enzymes toward sucrose was tested at pH 4.0 using an autolyzate of relatively high activity which was obtained from the cultures with dextrin.   As illustrated in Fig. 4, however, sucrose was not attacked even after the ihcubation of 40 hours when maltose was almost completely hydrolyzed. These observations indicate that this *Candia* yeast does not produce such invertase as is contained in *Saccharomyces* yeast.

    In order to make clear whether or not maltose and methyl-α-glucoside were hydrolyzed by the one and the same acid active enzyme the ratios of activity toward these two substrates were examined using three kinds of dry cell suspensions and one of the autolyzate. The results are summarized in Table 3, which shows that the activity ratios are almost constant among these enzyme preparations though the specific activity toward each substrate varied more or less considerably with
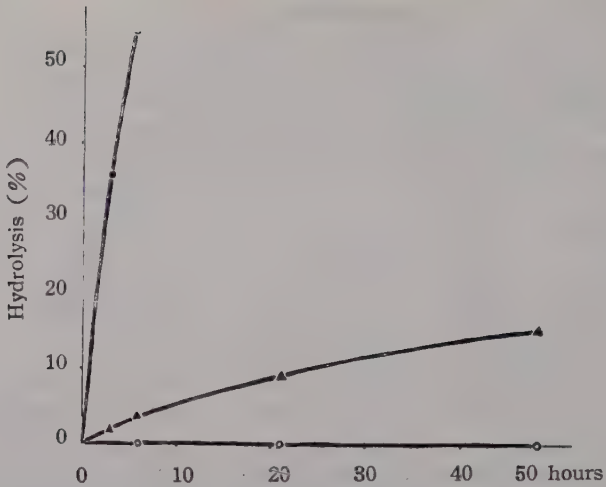
Fig. 4   Time-course of the hydrolysis of maltose (●), sucrose (○) and methyl–α–glucoside (▲) at pH 4.0 by the autolyzate obtained from the cells grown in a dextrin medium.

different enzyme preparations.   Hence it appears likely that maltose and methyl–α–glucoside would be hydrolyzed by a single enzyme.   This enzyme was thus named as acid α–glucosidase.

Table 3.   Activity of three different dry cell suspensions and of an autolyzate for the hydrolysis of maltose and methyl-α-glucoside at pH 4.0. The autolyzate was treated in two ways.

C-source used for culture	Weight of dry cells per ml. of digest, mgr. / Enzyme preparation \ Substrate		Reaction period, min.		Velocity constant, $10^4 \cdot k^*$		Activity ratio **	
			Maltose	Me-α-gl.	Maltose	Me-α-gl.	Maltose	Me-α-gl.
Dextrin	Dry cell suspension	0.5	480	1740	17.72	0.68	100	3.4
Me–α–gl.	Dry cell suspension	0.83	480	1740	6.85	0.27	100	4.0
Mannose	Dry cell suspension	5.5	480	1740	1.14	0.045	100	3.9
Dextrin	Autolyzate untreated (control)		180	1740	7.17	0.27	100	3.7
	Autolyzate heated at 45° for 1 hour		180	1170	6.50	0.25	100	3.8
	Autolyzate heated at 60° for 1 hour		180	1170	3.19	0.14	100	4.4

*   In the case of the dry cell suspensions the velocity constant was divided by mgr. of dry cells per ml. of digest.

**   Activity for maltose was taken as 100.

(3)   Trehalase.   The dry cell suspension of this *Candida* yeast grown in a mannitol medium contained a trehalase which hydrolyzed trehalose optimally at pH 5.2 (Fig 5).   The trehalase was contained in varying amounts in the cells grown with various other carbon sources.   In order to see whether this trehalase was identical either with the acid α–glucosidase or with any one of the neutral α–glucosidase group, activity ratios of all these enzyme preparations and the trehalase

toward four substrates (sucrose, maltose, methyl-α-glucoside and trehalose) were determined using three sorts of dry cell suspensions. Table 4 summarizes the results. Since, in the case of two dry cell suspensions which were obtained from the cells grown in the sucrose medium and in the mannitol medium respectively, the activity ratios toward trehalose and each of three substrates of the neutral α-glucosidase group varied remarkably, it may be sure that the trehalase differs from any one
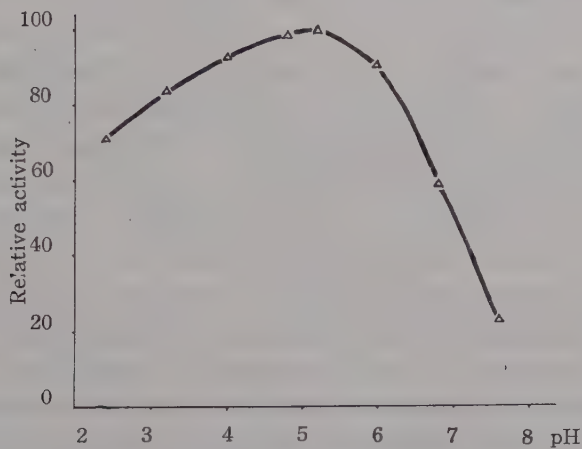
Fig. 5　Activity-pH-curves of the dry cell suspension obtained from the cells grown in a mannitol medium for the hydrolysis of trehalose. Period of incubation: 150 min.

of the neutral α-glucosidases. Similarly, the trehalase appears to be distinct from the acid α-glucosidase, because there is no parallelism in the activities for trehalose and

Table 4.　Activity of the neutral α-glucosidase group, the acid α-glucosidase and the trehalase contained in the three different sorts of dry cell suspensions.

Enzyme	Substrate	Reaction pH	Reaction period min.			Velocity constant, $10^4 \cdot k$			Activity ratio *		
			Mannitol	Sucrose	Dextrin	Mannitol	Sucrose	Dextrin	Mannitol	Sucrose	Dextrin
Neutal α-glucosidase group	Sucrose	6.6	1320	150		0.42	4.92		4	863	
	Maltose	6.6	1320	150		0.89	3.08		8	540	
	Me-α-gl.	6.1	1320	150		0.07	1.61		0.7	282	
Trehalase	Trehalose	5.2	150	1320	150	10.49	0.57	12.30	100	100	100
Acid α-glucosidase	Maltose	4.0	1320		150	1.51		22.50	14		183

*　Activity for trehalose was taken as 100.

maltose in the two dry cell suspensions which were obtained from mannitol culture and dextrin culture respectively.　Therefore it seems probable that the trehalase of this *Candida* yeast is a specific enzyme that hydrolyzes trehalose alone.

Discussion

Candida tropicalis var. *japonica* produces two sorts of maltose hydrolyzing enzymes, the one with the optimum pH at about 4.0 and the other with that at 6.6.　The latter enzyme seems to be, for its lability in the acid medium and its optimum pH value (Fig. 1). the same as or analogous to the enzyme which has heretofore been known as yeast maltase (12, 13).　Willstätter et al. (12) reported long ago that the yeast maltase could hydrolyse methyl-α-glucoside too.　Weidenhagen (14), who discovered that this enzyme could further hydrolyze sucrose, designated it as α-glucosidase and considered that the α-glucosidase hydrolyzes generally varicus holosides and heterosides with α-glucosidic linkage.　In this *Candida* yeast, however, the author postulated that the enzymes which hydrolyzed maltose, sucrose and methyl-α-glucoside optimally at pH 6.1 to 6.6 consisted of at least three distnct enzyme because the activity ratios of the enzyme preparation toward these three substrates fluctuated quite irregularly upon various treatments.　In 1940 Miwa and Toishi (15) observed that the ratio of the activity of yeast α-glucosidase toward maltose and methyl-α-glucoside fluctuated more or less significantly upon fractionation of the enzyme preparation and suggested that maltase and methyl-α-glucosidase of brewer's yeast might be separate enzymes.　Recently Lindegren and his associates (16, 17) reported that they could obtain from haploid *Saccharomyces* strains maltase, sucrase and methyl-α-glucosidase each of which acted specifically upon a corresponding single substrate.　In view of these findings it may be possible to infer that in our neutral α-glucosidase group of this *Candida* yeast at least three distinct enzymes may be involved, each acting exclusively upon sucrose, maltose and methyl-α-glucoside respectively.

On the contrary, the acid α-glucosidase may be regarded as an enzyme which hydrolyzed both maltose and methyl-α-glucoside, since the ratios of activity toward these substrates remained nearly constant in different enzyme preparations, as shown in Table 3.　However, this α-glucosidase did not act upon sucrose, a fact which is at variance with the Weidenhagen's theory.　In this respect it resembles rather the maltases found in *Schizosaccharomyces octosporus* (18) or *Escherichia coli* (19, 20). While Hofmann could not obtain a decisive evidence for the identity of maltase and methyl-α-glucosidase of the *Schizosaccharomyces* yeast (18), it appears of interest that the average activity ratio of the *Candida* acid α-glucosidase for the hydrolysis of maltose and methyl-α-glucoside, 100:3.9, is close to that of the *Schizosaccharomyces* yeast, 100:2.8, when calculated from the Hofmann's data.

Based on the observations that the ratio of activity toward maltose and trehalose differs between the enzyme preparation of baker's yeast and that of brewer's yeast

and that the stability of each enzyme preparation differs in respect of two substrates, Myrbäck and Örtenfeld (21) considered that maltase and trehalase were different enzymes. In the present work more reliable data were obtained for proving the non-identity of the trehalase with the acid α-glucosidase and also with each of the neutral α-glucosidase group of a *Candida* yeast strain (Table 4). It is worthy to note that the optimum pH of this *Candida* trehalase, namely about 5.2, was the same as well as that of the trehalase found by Lukes and Phaff (9) in the cells of another strain of *Candida tropicalis* while it differs from that of the trehalase of the brewer's yeast which lies at about 7.2 (21).

The experimental data obtained in the present work are strongly suggestive of the absence of β-h-fructosidase in the cells of *Candida tropicalis* var. *japonica* (Fig. 2 and 4). In this respect the constitution of the carbohydrase system of this *Candida* yeast seems of particular interest.

Summary

Candida tropicalis var. *japonica* was proved to produce following three sorts of α-glucosidases.

(1) Neutral α-glucosidase group. The enzyme included in this group hydrolyzed sucrose, maltose and methyl-α-glucoside optimally at pH 6.1 to 6.6, but not below pH 4.0. In this group at least three different enzymes seem to de involved, but the separation of the enzymes was not successful.

(2) Acid α-glucosidase. This enzyme hydrolyzed both maltose and methyl-α-glucoside optimally at about pH 4.0, but not sucrose at all. The activity ratio of this enzyme toward these two substrates was about 100 : 3.9.

(3) Trehalase. This enzyme hydrolyzed trehalose alone optimally at pH 5.2 and was proved to differ from any other α-glucosidases mentioned above. β-h-Fructosidase appeared to be absent in this yeast strain.

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Electron-microscopical Study on Fine Structures
of Diatom Frustules XIV
Observation on the genus *Chaetoceros*[1]

by Haruo OKUNO*

奥野春雄：　電子顕微鏡による珪藻殻微細構造の研究 XIV
キートセロス属についての観察

Chaetoceros affinis Lauder (Pl. I), Hustedt, Kieselalg. **1**: 695, fig. 396 (1930); Mills, Index Diat. 370 (1933).

L. M. S.[2] (Fig. 1)　Chains straight.　Frustules in broad girdle view oblong, about 13-23 (9-30)μ broad.　Apertures between frustules narrow lanceolate, slightly constricted in the middle.　Valves (v) elliptic, each of the terminal frustules possessing short central spine (cs).　Mantles (m) high, suture (s) between mantle and girdle slightly constricted.　Inner setae delicate, arising from the angles of valves, coalescing at the base with the setae of the opposite frustule.　Terminal setae robust, strongly divergent.

E. M. S.[3] (Fig. 2–5)　In the present research, frustules and setae of the terminal cells were observed.　Frustules indistinctly ribbed.　On the valve, ribs (r) are arranged in radiating rows, about 4 in 1 μ, often divaricate and anastomose.　On the mantle, ribs are arranged in longitudinal rows about 4 in 1 μ.　Frustule pores are round or angular holes (h), about 100 mμ in diameter, about 2–4 in 1 μ, scattered in the indistinct furrows between ribs.　Besides holes, small pits (p, minute, incomplete holes with somewhat thick closing membranes) about 10 mμ in diameter are also scattered on the frustule.　Central spine (cs) of the terminal valve is elliptic at the base, and perforated by a flat canal with a basal opening (ocs) about 900 mμ long aud 50 mμ broad.　In the present specimen, the central spine, at its end, splits

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* Botanical Laboratory, Kyoto University of Industrial Arts and Textile Fibers, Kitaku, Kyoto.
京都工芸繊維大学繊維学部植物学研究室.

1) Hitherto, so far as I know, the following species were researched with the electron microscope:　*Ch. criophilum* (Okuno, 1952, 1954), *Ch. dichaeta* (Okuno, 1952, 1953, 1954), *Ch. didymus* (Desikachary and Bahadur, 1954), *Ch. Eibenii* (Desikachary, 1954), *Ch. Lorenzianus* (Desikachary and Bahadur, 1954), *Ch. paradoxum* (Desikachary and Bahadur, 1954), *Ch. pervianus* (Desikachary, 1954).　Besides these species, some others may have been treated in J. G. Helmcke and W. Krieger, Diatomeenschalen in elektronen-mikroskopischen Bild. I, II (1953, 1954), but I have not yet been able to see the publication.

2) L. M. S.:　Light-microscopic structure.

3) E. M. S.:　Electron-microscopic structure.

into two lips.   Besides the central spine, many sharp spinules (*spn*) about 0.3–0.4 μ long are scattered on the valve surface.   Terminal seta hollow, and its wall seems to be perforated by somewhat coarse pores.

Habitat : Marine plankton.   Shimonoseki, Yamaguchi Prefecture (Okuno, No. m859. May, 1953.   Collected by H. Maeda).   Kariya, Awaji Island, Hyôgo Prefecture (Okuno, No. m989. Aug. 1954).

Chaetoceros atlanticus Cleve (Pl. II), Hustedt, Kieselalg. **1**: 641, figs. 363, 364 (1930) ; Mills, Index Diat. 372 (1933).

L. M. S. (Figs. 1, 2)   Chains straight, not twisted.   Frustules subcylindrical, oblong, 20–42 μ broad in broad girdle view.   Valves elliptic, with a central spine (*cs*) about 4–6 μ long and 500–600 mμ in diameter.   Apertures hexagonal, rounded at corners.   Mantles low, usually less than one–third of the height of frustule.   Girdle well developed.   Suture between mantle and girdle distinct.   Setae arising slightly within the apical ends, basal part somewhat thin, after crossing thickened, later tapered toward the ends.   Inner setae almost straight.   Terminal setae slightly thicker and longer or shorter than the inner setae, strongly divergent outwards, sometimes bent towards pervalvar axis further out.

E. M. S. (Figs. 3–7)   Valve surface has delicate radial ribs (*r*) about 3–5 in 1μ, and between the ribs scattered with round holes (*h*) about 60–100 mμ in diameter, about 6–8 in 10μ.   Besides these holes, pits (*p*) about 20–30 mμ in diameter are sometimes densely distributed on the valve surface.   Central spine round, hollow, about 4–6 μ long about 550–600 mμ in diameter ; its wall probably non-porous.   Mantle line (*ml*) thickened, rarely armed with small numbers of fine spinules (*spn*) about 0.6–1 μ long.   Mantle about 200–400μ high, scattered with round holes about 100–150μ in diameter, and apparently ribless.   The girdle was impenetrable to the electron beam, and no fine structures could be revealed.   Setae hollow, angular[4], with thickened and spinulated corners.   Side wall of the seta thin, perforated with coarse pores about 2–2.5 in 1 μ, arranged in a longitudinal row.   The pore rounded rectangular, incompletely locular with the inward projection of side wall.   At its outside, the incomplete loculus (*il*) is half closed by a more or less netveined sieve membrane (*sm*), leaving several rounded or horse-shoe shaped slit-like sieve pores (*sp*).   Walls of setae are also scattered with pits about 50 mμ in diameter.

Habitat : Marine plankton.   Abundant in our Northern Pacific collections from 48°48′–52°30′ N, 162°58′–179°50′ E (Okuno, No. m698–741. June–July, 1952. Collected by Captain S. Sakai on the researching boat "Ten-yô-Maru" and Mr. H. Maeda on the guard boat "Syunkotu-Maru").

Chaetoceros danicus Cleve (Pl. III), Hustedt, in A.   Schmidt, Atlas Diat. Pl. 342, fig. 9 (1921) ; Mills, Index Diat. 380 (1933).

L. M. S. (Flg. 1)   Cells usually solitary, sometimes in short chains with very

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4) According to Hustedt, octangular [Hustedt (1930), 641].

small apertures. Valves almost flat, broad elliptical, 12–20 (7–20) μ long, 10–17 μ broad. Mantles somewhat high. Suture between mantle and girdle distinct. Girdle narrow, about 1.5–2 μ broad. Setae thin, almost horizontal, divergent in acute angles on both valves, and form a cross in valve view [cf. Hustedt (1930), 660, fig. 373 a, b].

E. M. S. (Figs. 2–5) Valves usually ribless, rarely with indistinct ribs (r). Central pores (cp) of the valve more or less linear, about 200–300 mμ long and 100–130 mμ broad. Holes (h) round, about 50–80 mμ in diameter, irregularly scattered both on valve and mantle. On the valve, the holes denser at the margin than in the center. Valve surface without spinules. Setae round or angular, about 2–2.7 μ in diameter. The base of seta (bs) swollen in bulb-form. Basal opening of the seta, through which the cavity of seta communicates with the cell cavity, is elliptic, about 5 μ long and 3 μ broad. Wall of seta is perforated with round to rectangular holes about 50–80 mμ in diameter or about 100 mμ long and 50–80 mμ broad. Holes of the seta arranged in circular and longitudinal rows about 5–6 in 10 μ. Near the end, the seta is armed with fine spinules about 0.8–1.5 μ long.

Habitat: Marine plankton. Suma and Kariya, Hyôgo Prefecture (Okuno, No. m948–Suma. March, 1954. No. m989–Kariya. Aug. 1954).

Chaetoceros didymus Ehrenberg (Pl. IV, Fig. 2), Hustedt, Kieselalg. **1**: 688, figs. 390, 391 (1930); Mills, Index Diat. 383 (1933); Desikachary and Bahadur, Journ. Sci. & Indust. Res. **13** B: 92, fig. 1 (4, 10, 11) (1954)

L. M. S. Chains straight, with somewhat broad apertures constricted in the middle. Frustules in broad girdle view quadrangular, about 10–25 (10–40) μ broad. Valves (v) elliptic, with a hemispherical protuberance (pr) in the center. Mantle (m) narrow. Girdle more or less broad. Setae arising from corners, angular, crossing at their bases, on the edges armed with spinules (spn). Terminal setae long, divergent, slightly thicker than the others.

Habitat: Marine plankton. Kariya, Awaji Island, Hyôgo Prefecture (Okuno, No. m989. Aug. 1954).

var. **protuberans** (Lauder) Gran and Yendo (Pl. IV, Figs. 3, 4), Hustedt, Kieselalg. **1**: 690, fig. 392 (1930)

Terminal setae distinctly thicker and more strongly divergent than in type, and convex toward outside. Inner setae, having more or less long basal parts, cross far out from the chain.

Habitat: Shinwakanoura, Wakayama Prefecture (Okuno, No. m618. June, 1952).

var. **anglica** (Grunow) Gran (Pl. IV, Fig. 1), Hustedt, Kieselalg. **1**: 690, fig. 393 (1930)

Inner setae, having long basal parts, cross further out from the chain than in type and var. *protuberans*.

Habitat: Kariya, Awaji Island, Hyôgo Prefecture (Okuno, No. m689. Aug. 1954).

E. M. S The three forms, *Ch. didymus*, var. *protuberans* and *anglica* are the

same in their electron-microscopic fine structures. Frustule ribs (r) very thin, more or less distinctly visible on the protuberance (pr) and its environs where the holes (h) are sparse; on the other parts where holes are dense, the ribs indistinct. Holes round to angular, 4-6 in $1\,\mu$; on the valve about 60–100 mμ in diameter, arranged in radial rows, and usually somewhat sparse near the protuberance and the base of seta. On the mantle (m) and girdle, holes are arranged in longitudinal rows. By the present electron microscopy, intercalary bands (ib)[5] were found in the three forms. The imbrication line (iml) of the intercalary band distinct. Intercalary bands are collar-shaped, circular (or semicircular?), about 3.5–5 μ broad, and finely porous with holes. Holes round to angular, about 50–80 mμ in diameter, arranged in longitudinal rows about 5–6 in $1\,\mu$. Holes are a little smaller than those of the valve and mantle. Terminal seta quadrangular (or pentagonal?), with one or two rows of holes on each side about 2-4 in $1\,\mu$. Holes elliptic, about 100–250 mμ long and 60–150 mμ broad. Corners of seta armed with spinules about 150–200 mμ long, 5–6 in $10\,\mu$. Desikachary and Bahadur published three electron micrographs of *Ch. didymus* in Journ. Sci. & Indust. Res. **13** B: 93, fig. 1 (4, 10, 11). Their figure 4 shows a seta with rounded holes arranged in circular and longitudinal rows about 6–7 in $1\,\mu$. This is quite different in fine structure from that of the present specimens. The shape and arrangement of the holes in their figure 4 are rather similar to those of *Ch. danicus* here researched (Pl. III, fig. 2).

Chaetoceros distans Cleve (Pl. V), Hustedt, in A. Schmidt, Atlas Diat. pl. 337, figs. 3-6, pl. 338, fig. 8 (1921); Mills, Index Diat. 384 (1933).

L. M. S. (Fig. 1) Chains straight or more or less twisted. Frustules in broad girdle view rectangular, $10–26\,\mu$ broad, with slightly projecting corners. Valve surface elliptic, rarely almost circular, slightly convex in the middle. Apertures very large, slightly constricted in the middle. Inner setae thin, with long basal part, toward the ends curved to one end of chain. Terminal setae thick, more or less divergent, armed with fine spinules. Chromatophore one per cell, central or pressed against one valve. No resting spores observed in the present specimens. Light-microscopically, the present species has a close resemblance to *Ch. laciniosus* Schütt. Hustedt [Kieselalg. **1**: 701 (1930)] and Mills [l. c., 384, (1933)] put the former in synonyms of the latter, but the present specimens had, as a distinct character of *Ch. distans*, one chromatophore per cell. (In *Ch. laciniosus*, chromatophores two per cell.) Kokubo [Plank. Diat. 184 (1955)] distinguished *Ch. distans* from *Ch. laciniosus* by its spinulated resting spores, and also Ikari [Bot. Mag. Tokyo **42**: 254 (1928)] by its longer basal part of inner setae. Thus, I identify the present specimens as *Ch. distans*.

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5) Hitherto known in *Ch. costatus* [Hustedt (1930), 699, fig. 399 d; Cupp (1943), 127, fig. 79], *Ch. denticulatum* [Ikari (1928), 249, fig. 3], *Ch. Eibenii* (Cupp, l. c., 7, fig. C8), *Ch. Okamurai* (Ikari, l. c., 249), and *Ch. rostratum* (Ikari, l. c., 249, fig. 3).

E. M. S. (Figs. 2–6) Frustules have somewhat thick, linear ribs (*r*) on valve (*v*), mantle (*m*) and girdle (*g*). Valve has a flat, elliptic central area (*ca*) about 1.3–6.5 μ long and 1–5 μ broad, which is usually hyaline, rarely scattered with holes. On valve surface, the ribs about 100–300 mμ broad, radiating from the central area about 2–3 in 1μ, and often divaricating several times. On mantle, the ribs arranged in longitudinal rows, about 2–4 in 1 μ. On girdle, the ribs thin, about 80 mμ broad, and arranged in longitudinal rows about 7 in 1 μ. The mantle line (*ml*) thick, rim-like, about 200 mμ broad. Frustules with or without holes. Holes (*h*) round, about 30–70 mμ in diameter, scattered sparsely on both ribs and furrows of valve and mantle. In the present specimens, holes were not found on the girdle. Setae hollow, round (sometimes slightly angular?), about 0.8–1.8 μ in diameter, armed with spinules (*spn*). Wall of the inner seta distinctly perforated with round to oblong holes about 15–30 mμ in diameter. Holes of the seta occur about 12 in 1 μ, and are arranged in circular and longitudinal rows.

Habitat: Marine plankton. Hamazume, Kyoto Prefecture (Okuno, No. m360. Aug. 1949). Maizuru, Kyoto Prefecture (Okuno, No. m433. Aug. 1949. Collected by H. Maeda). East China Sea: 31°54′–32°54′ N, 123°26′–124°39′ E (Okuno, No. m924–933. Jul.-Sept. 1953. Collected by H. Maeda). Minato, Awaji Island, Hyôgo Prefecture (Okuno, No. m1020. Aug. 1955. Collected by Y. Funakoshi).

Chaetoceros Lorenzianus Grunow (Pl. VI), Mills, Index Diat. 390 (1933); Cupp, Bull. Scrips Inst. Ocean. **5**: 118, fig. 71 (1943)

L. M. S. (Figs. 1, 2) Chains straight. Frustules in broad girdle view rectangular, about 30 (10–80) μ broad, with slightly elevated center and apical ends. Apertures elliptical to linear, slightly constricted in the middle. Valves elliptic with slightly elevated center and apical ends. Suture between mantle and girdle distinct. Setae strong, with a short basal part, fused adjacently only at point; the wall distinctly punctate. Terminal setae shorter and somewhat thicker than others, more or less divergent.

E. M. S. (Figs. 3–7) Valve (*v*) has an elliptic hyaline central area (*ca*) about 2 μ long and 1.2 μ broad. Ribs (*r*) distinct, radiate, somewhat thick, irregularly divaricate and anastomose; about 100–300 mμ broad, and occurring about 3 in 1μ at the valve margin. At the base of seta, ribs convergent. Holes (*h*) indistinct, about 30–40 mμ in diameter, scattered on ribs and rarely on furrows. Judging from the electron images, most of the holes may be the pits (*p*). Spinules (*spn*) about 300–500 mμ long rarely occur on ribs of the valve. Mantle line (*ml*) thickened, about 0.5 μ broad. Ribs on the mantle (*m*) longitudinal, about 3 in 1 μ. Holes and pits are sparsely scattered. Girdle (*g*) ribless, scattered with indistinct holes and pits. In Fig. 4, the imbrication zone (*iz*) between mantle and girdle is clearly shown. Setae rectangular, about 1.5μ in diameter at the base, and 2.5–3μ at other part. Wall of setae perforated by a row of coarse holes. Holes elliptic to oblong, 1–1.2 μ long and 0.5–0.7 μ broad, occurring about 5–7 in 10 μ. Corners of setae

armed with minute spinules.

Habitat: Marine plankton. Off Yosa Peninsula. 36°3.2′ N; 135°24′ E (Okuno, No. m454. Jul. 1950. Collected by H. Maeda). Kariya and Sumoto, Awaji Island, Hyôgo Prefecture (Okuno, Nos. m989, m995. Aug. 1954).

Chaetoceros decipiens Cleve (Pl. VII), Hustedt, Kieselalg. **1**: 675, fig. 383 (1930); Mills, Index Diat. 381 (1933).

L. M. S. (Figs. 1, 2) Light-microscopically, similar to *Ch. Lorenzianus*, but distinguishable from it by the longer fused part of the setae. The fused part is about two or three times as long as the diameter. Punctation of setae is more indistinct than in *Ch. Lorenzianus*.

E. M. S. (Figs. 3–8) Ribs very weak, scarcely visible, sometimes may be absent. Holes (*h*) round, about 70–100 mμ in diameter, 4–5 in 1 μ, arranged in radial rows common to valve (*v*) and mantle (*m*). Radial rows of holes about 4–5 in 1 μ. Mantle- (*ml*) and girdle lines (*gl*) distinct. Holes on the girdle (*g*) about 4–5 in 1 μ, arranged in longitudinal rows about 4–5 in 1 μ. Setae rectangular, about 1.7–2 μ in diameter; wall of seta has coarse elliptic to rectangular holes about 0.7–2 μ long and 0.4–0.5 μ broad, which occur about 7–10 in 10 μ and are arranged in a longitudinal line. Spinules (*spn*) of setae delicate. The figures in Bot. Mag. Tokyo **63**: 99, pl. 2, figs. 2, 2′ (Okuno, 1950) and figs. 1 (1, 2) in Journ. Sci. & Indust. Res. **13** B: 92 (Desikachary and Bahadur, 1954) which are described by the authors as *Ch. Lorenzianus* show the same fine structure as in *Ch. decipiens* here researched. On the other hand, by the present research, *Ch. Lorenzianus* was found to have a quite different fine structure as detailed above. Thus, I now think these figures by Okuno, Desikachary and Bahadur should be shown under the name of *Ch. decipiens*.

Habitat: Marine plankton. 29° N; 135° E (Okuno, No. m980. Dec. 1950. Collected by R. Marumo). Kariya, Awaji Island, Hyôgo Prefecture (Okuno, No. m989. Aug. 1954).

Chaetoceros pervianus Brightwell (Pl. VIII), Hustedt, Kieselalg. **1**: 671, figs. 380–381 (1930); Mills, Index Diat. 393 (1933); Desikachary, Mikrosk. **9**: 174, figs. 24, 26 (1954)

L. M. S. (Fig. 1) Cells usually solitary. Frustules 12–26 (10–30) μ broad in broad girdle view. Valve surfaces elliptic, dimorphic; the upper rounded, the lower flat. Suture between mantle and girdle deep, groove-like Setae of upper valve originate near center, and those of lower valve originate near margin. Setae thick, hollow, four-sided; walls transversely costated, armed with spinules on corners.

E. M. S. (Figs. 2–8) Frustules ribless, very rarely with weak ribs (*r*), radiate on valve and longitudinal on mantle. Mantle line slightly thickened. Holes (*h*) round, 40–70 mμ in diameter, sparse, irregularly scattered on valve, mantle and girdle. In ribbed frustules, holes distributed on furrows. Holes sometimes denser around the base of setae. Setae 2.5–4.2 (2–6) μ thick, rectangular, one side about

2–2.5 μ broad, basal part 2.5-3.3 μ thick, end pointed and closed. Spinules (*spn*) of setae distinct. Upper and lower setae are the same in fine structure. Seta walls have about 2 transverse costae (*c*) in 1 μ, and between every two costae about 5 (in 1μ 5-9) transverse rows of holes (*h*). Holes round, about 50–70mμ in diameter, occurring 7-9 in 1 μ. Central spine (*cs*) of the lower valve about 0.5–1μ in diameter and 2-3μ long. Desikachary published two electron micrographs of the present species [Desikachary, l. c., 177, figs. 24, 26 (1954)]. His fig. 24 shows the transverse costae of the lower seta, but the holes of seta were not revealed, and fig. 26 shows rounded, irregularly scattered holes on ribless mantle. These features quite agree with those in my present specimens.

Habitat: Marine plankton. Hamazume, Kyoto Prefecture (Okuno, No. m354. Aug. 1949). Akashi, Hyôgo Prefecture (Okuno, No. m393. Dec. 1949). 39° N; 153° E (Okuno, No. m972. Aug. 1950. No. m977. Nov. 1952. Collected by R. Marumo). Minato, Awaji, Hyôgo Prefecture (Okuno, No. m991. Aug. 1954. Collected by Y. Funakoshi).

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Desikachary, T. V. and Bahadur, K., Journ. Sci. & Indust. Res. 11 B: 491 (1952). 13 B: 92. 240 (1954). ———— & ————, Trans. Amer. Microsc. Soc. 73: 274 (1954). Desikachary, T. V., Amer. Journ. Bot. 41: 616 (1954). ————, Mikrosk. 9: 168 (1954). Fott, B. and Rozsival, M., Stud. Bot. Čechosl. 11: 262 (1955). Helmcke, J. G. and Krieger, W., Ber. Deut. Bot. Ges. 64: 29 (1951). Okuno, H., Rev. Cytol. et Biol. Végét. 15: 237 (1954). ————, Bot. Mag. Tokyo 68: 125 (1955). ————, Trans. Proc. Palaeont. Soc. Jap. N. S. 19: 51 (1955). ————, Keisô ni mirareru gokubi no Kôzô (Fine structures of diatom frustules). Sôbi (Magazine of Art-Design, Kyoto) 21: 1 (1955).
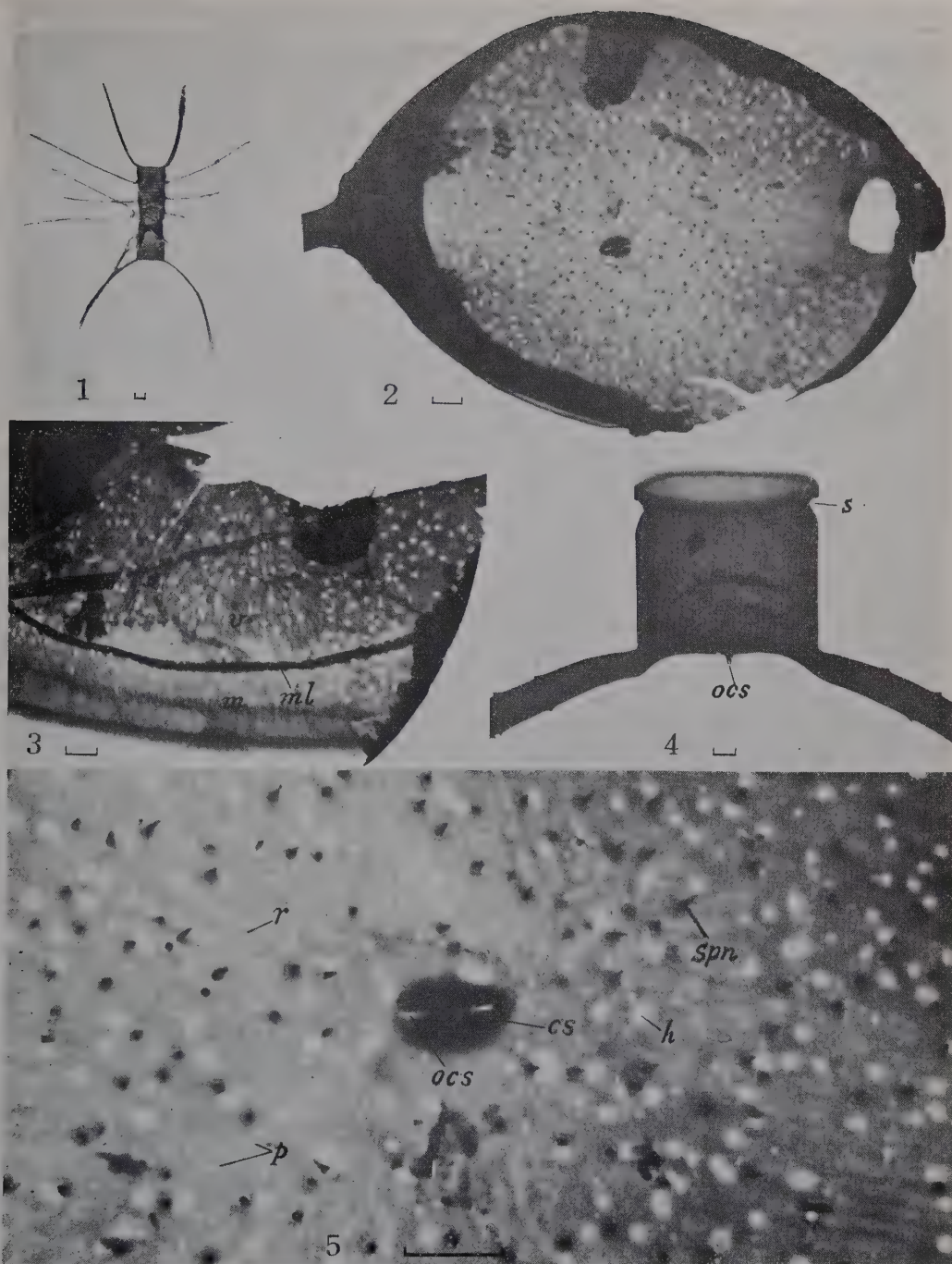
Figs 1–5, *Chaetoceros affinis*.   1, Entire chain in broad girdle view.   2, Terminal valve.
3, Portion of valve and mantle.   4, Terminal frustule; note the central spine with a slit-like
opening.   5, Central portion of the valve shown in fig. 2.   1–3, 5, Shimonoseki.   4, Kariya.
(1, Light micrograph.   Scale: 10μ. 2–5, Electron micrographs. Scales: 1μ.)

H. Okuno: Fine structure of diatom frustules
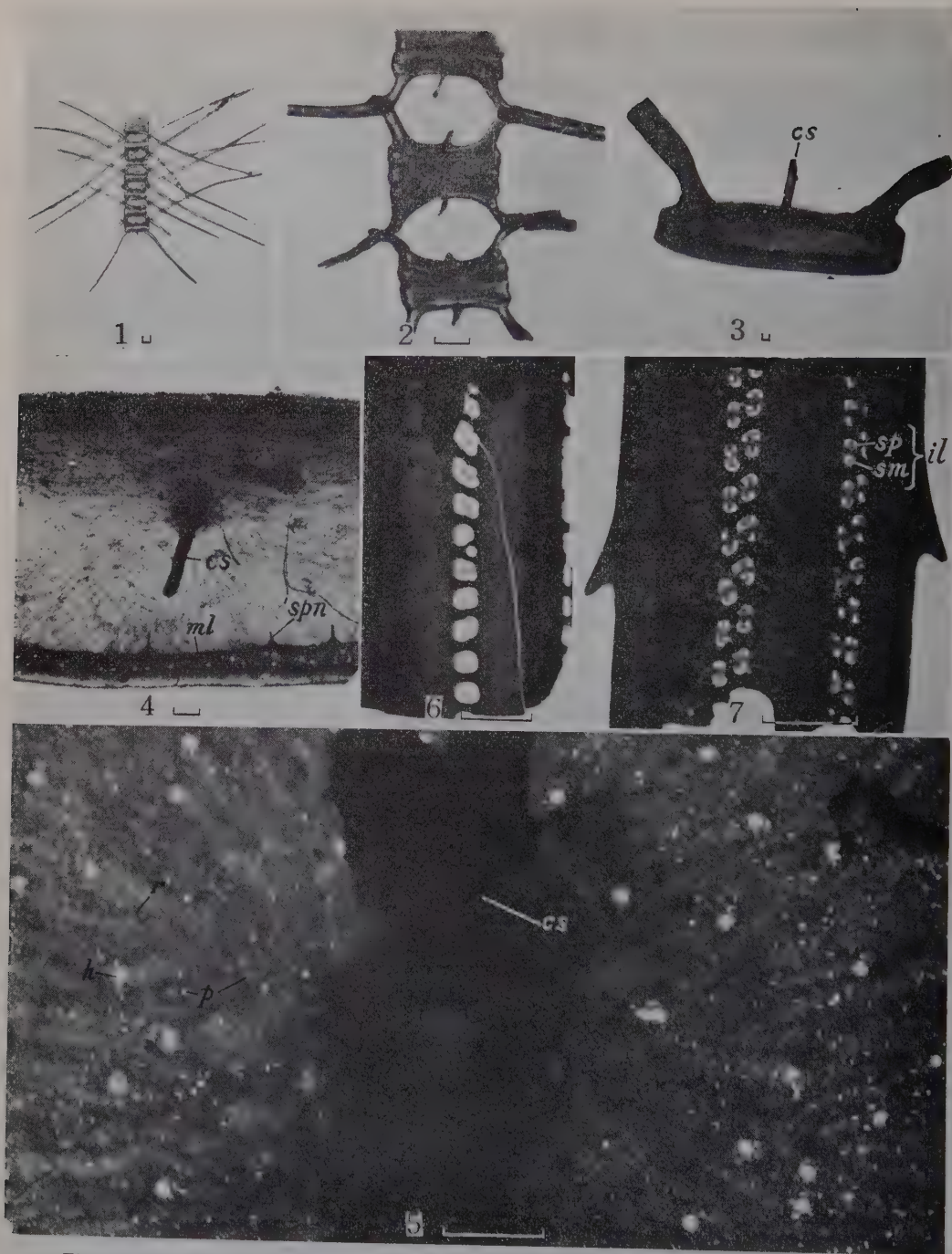
Figs. 1–7, *Chaetoceros atlanticus*.   1, Chain in broad girdle view.   2, Part of a chain, showing frustules with central spines and two setae.   3, Isolated single valve with a central spine.   4, Portion of a valve, showing central spine and spinules on mantle line.   5, Portion of a valve, showing holes, pits, and radial ribs.   6, 7, Sections of setae, showing incomplete loculi.   1–7, North Pacific Ocean.   (1, 2, Light micrographs, Scales: 10μ.   3–7, Electron micrographs. Scales: 1μ.)

H. Okuno: Fine structure of diatom frustules
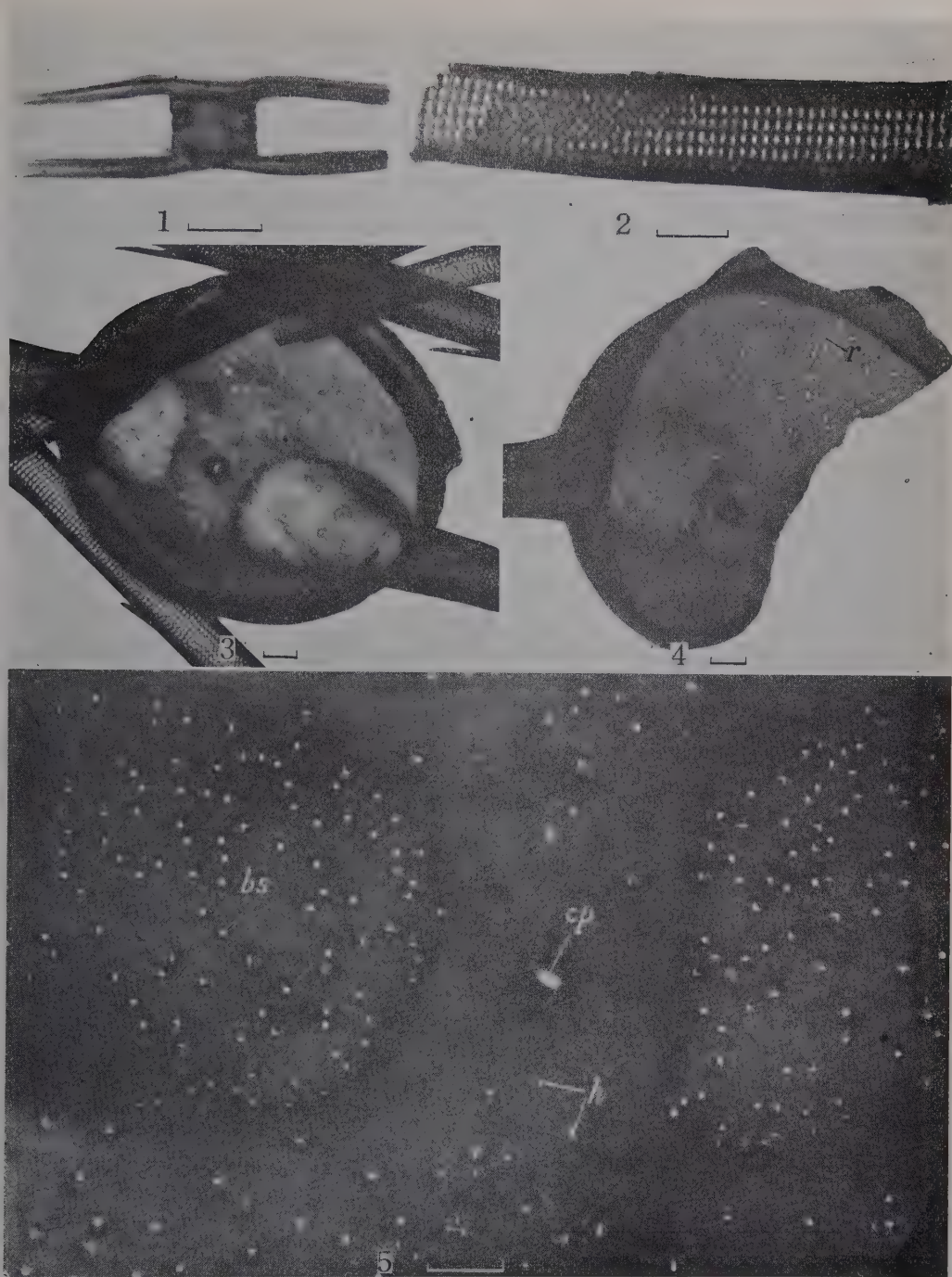
Figs. 1–5, *Chaetoceros danicus.*   1, Single cell in broad girdle view.   2, Section of a seta, showing round holes on the wall.   3, Valve viewed from outside.   4, Portion of a valve viewed from inside; note the indistinct radial ribs.   5, Central portion of a valve, showing a central pore, holes, and bulbar bases of setae.   1–4, Suma.   5, Kariya.   (1, Light micrograph. Scale: 10μ. 2–5, Electron micrographs. Scales: 1μ.)

H. Okuno: Fine structure of diatom frustules
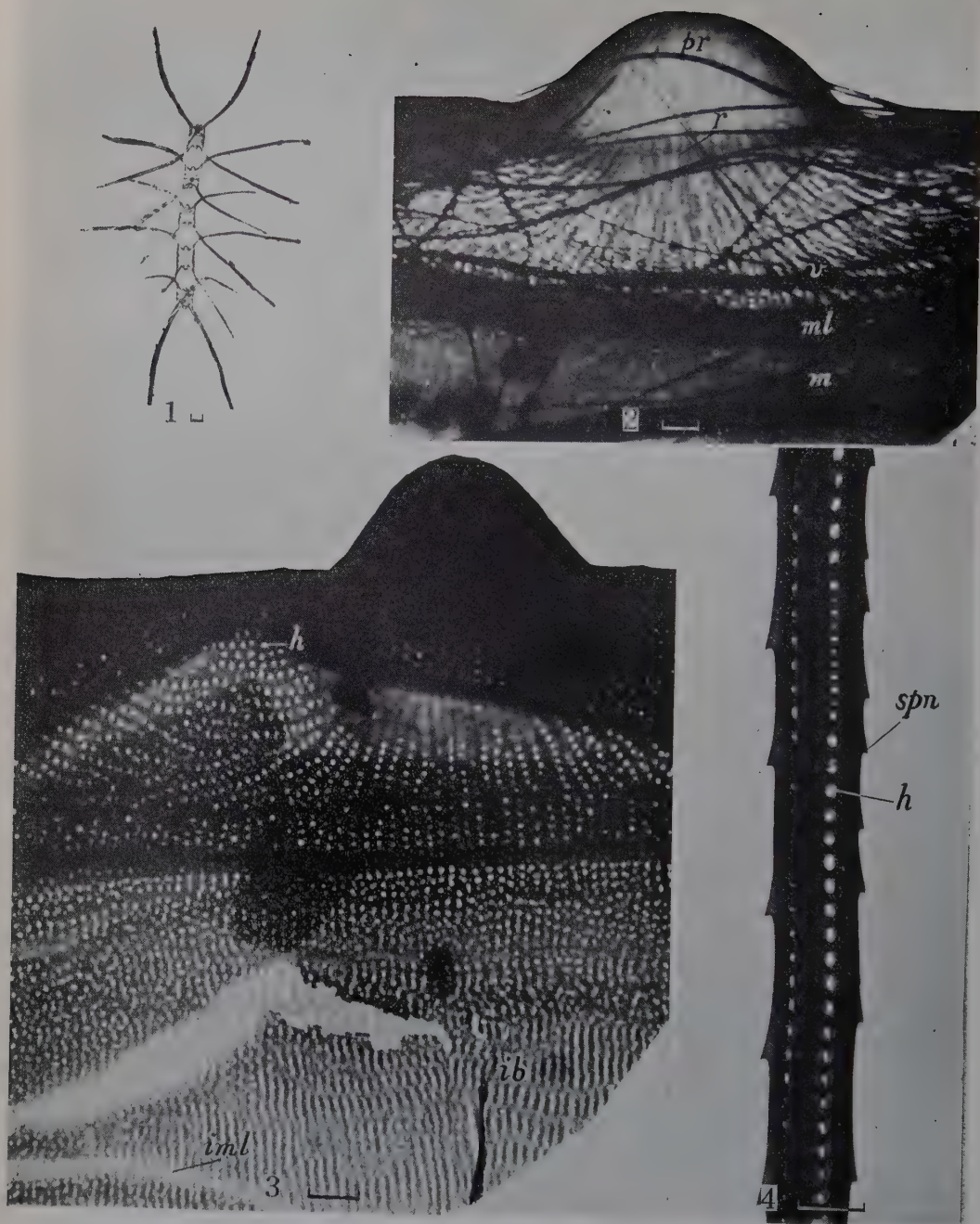
Figs. 1–4, *Chaetoceros didymus* and varieties.　　1, var. *anglica;* entire chain.　　2, Type; a portion of valve and mantle.　　3, 4, var. *protuberans.*　　3, Portion of a frustule, showing fine structure of valve and intercalary bands.　　4, Section of a seta, showing holes and spinules. 1, 2, Kariya.　3, 4, Shinwakanoura. (1, Light micrograph.　Scale: 10μ.　2–4, Electron micrographs. Scales: 1μ.)

H. Okuno: Fine structure of diatom frustules
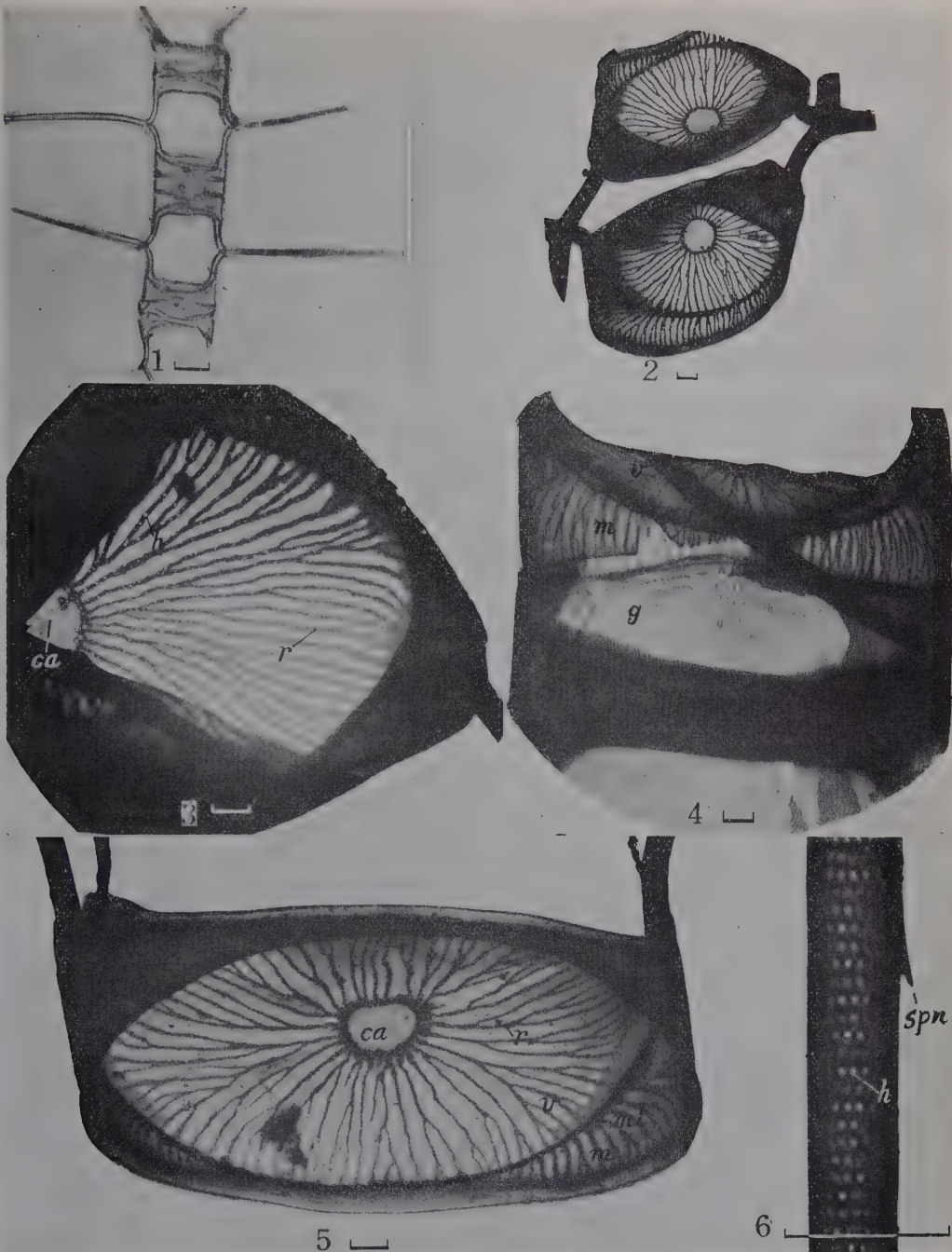
Figs. 1–6, *Chaetoceros distans*.   1, 2, Parts of chains.   3, Portion of a valve,   4, Frustule in girdle view; note the finely ribbed girdle. 5, Isolated single valve viewed from outside. 6, Section of a seta, showing holes and a spinule.   1, 3, 6, East China Sea.   2, Minato.   4, 5, Hamazume. (1) Light micrograph. Scale: 10μ. Electron micrographs. Scales: 1μ.)

H. Okuno: Fine structure of diatom frustules
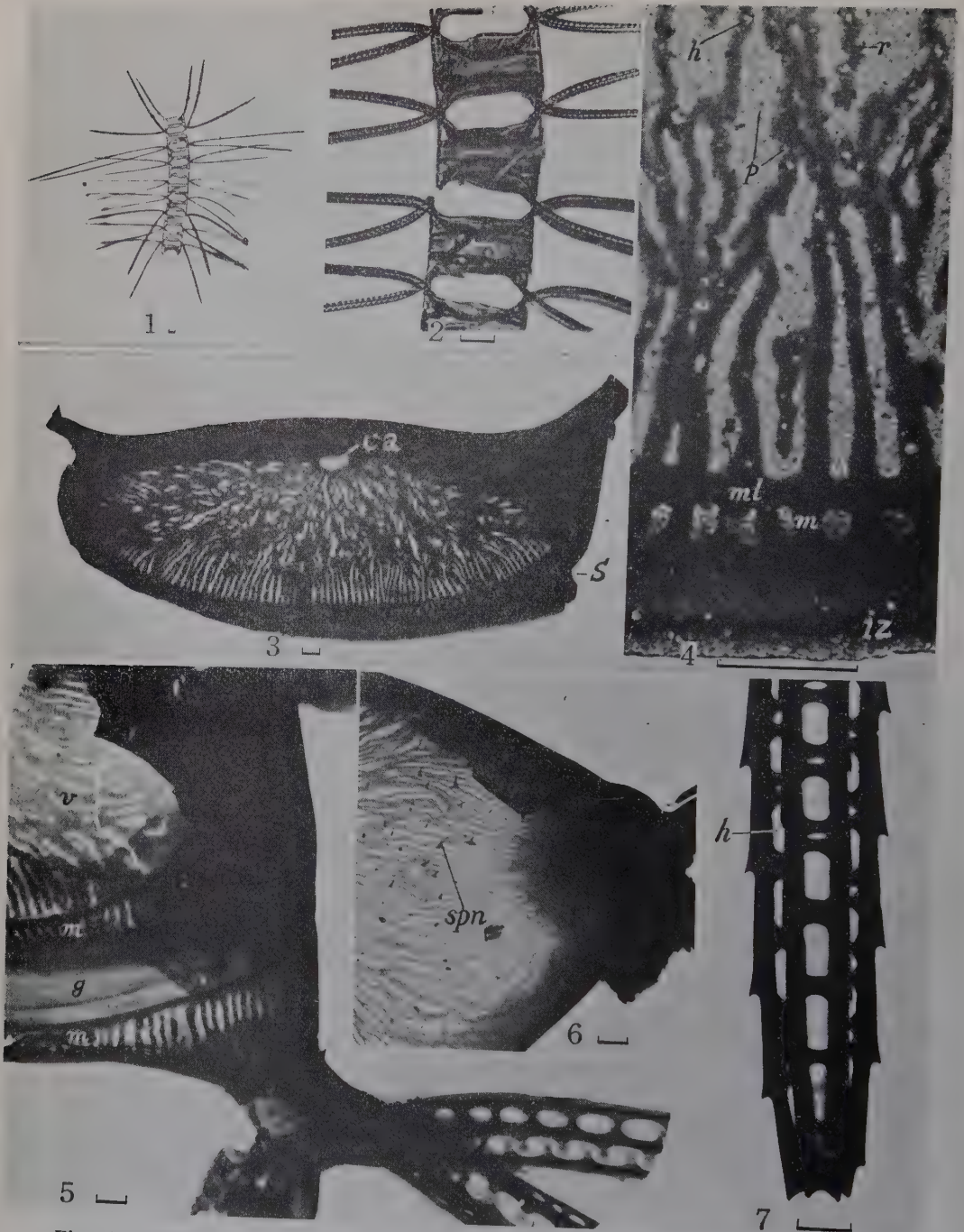
Figs. 1-7, *Chaetoceros Lorenzianus*. 1, Entire chain. 2, Part of a chain, showing holes on setae. 3, Isolated single valve, showing a central area, fused ribs. 4, Portion of a valve, showing dense holes and pit. 5, Girdle view of frustule with setae. 6, Portion of a valve, showing spinules. 7, Terminal portion of a seta, showing holes and spinules. 1, 2, Sumoto. 3, 5-7, Kariya. 4, Yosaumi. (1, 2, Light micrographs. Scales : 10μ. 3-7. Electron micrographs. Scales : 1μ.)

H. Okuno : Fine structure of diatom frustules
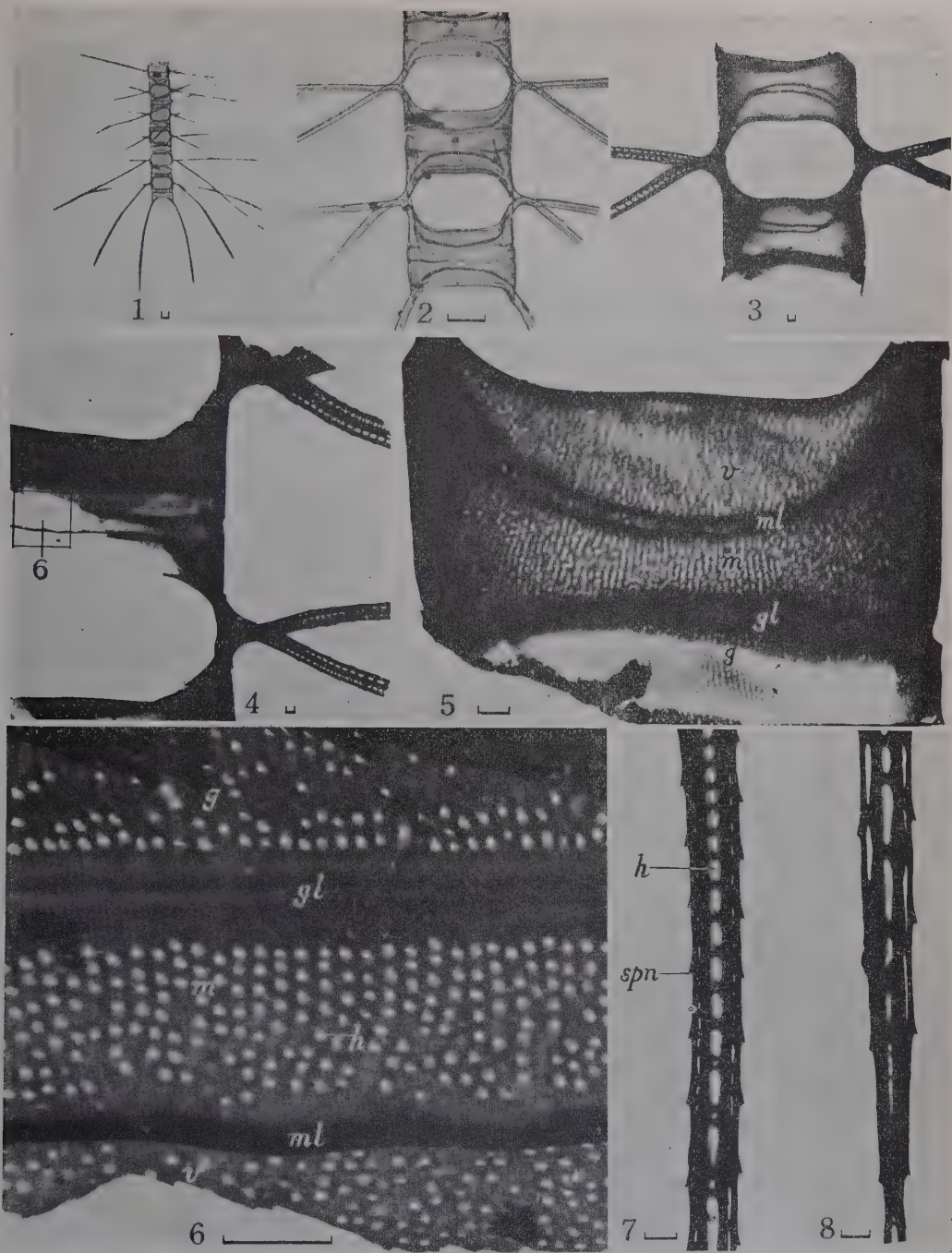
Figs. 1-8, *Chaetoceros decipiens*.   1, Chain.   2, Part of the chain in fig. 1.   3, 4, Parts of chains.   (Note the long fused basal part of setae in figs. 2, 3.)   5, Part of a frustule.   6, Portion of a frustule in fig. 4, showing holes, mantle- and girdle lines.   7. Section of an inner seta.   8, Terminal portion of an inner seta.   1, 2, 29°N; 135°E.   3-8, Kariya.   (1, 2, Light micrographs. Scales: 10μ.   3-8, Electron micrographs.   Scales: 1μ.)

H. Okuno: Fine structure of diatom frustules
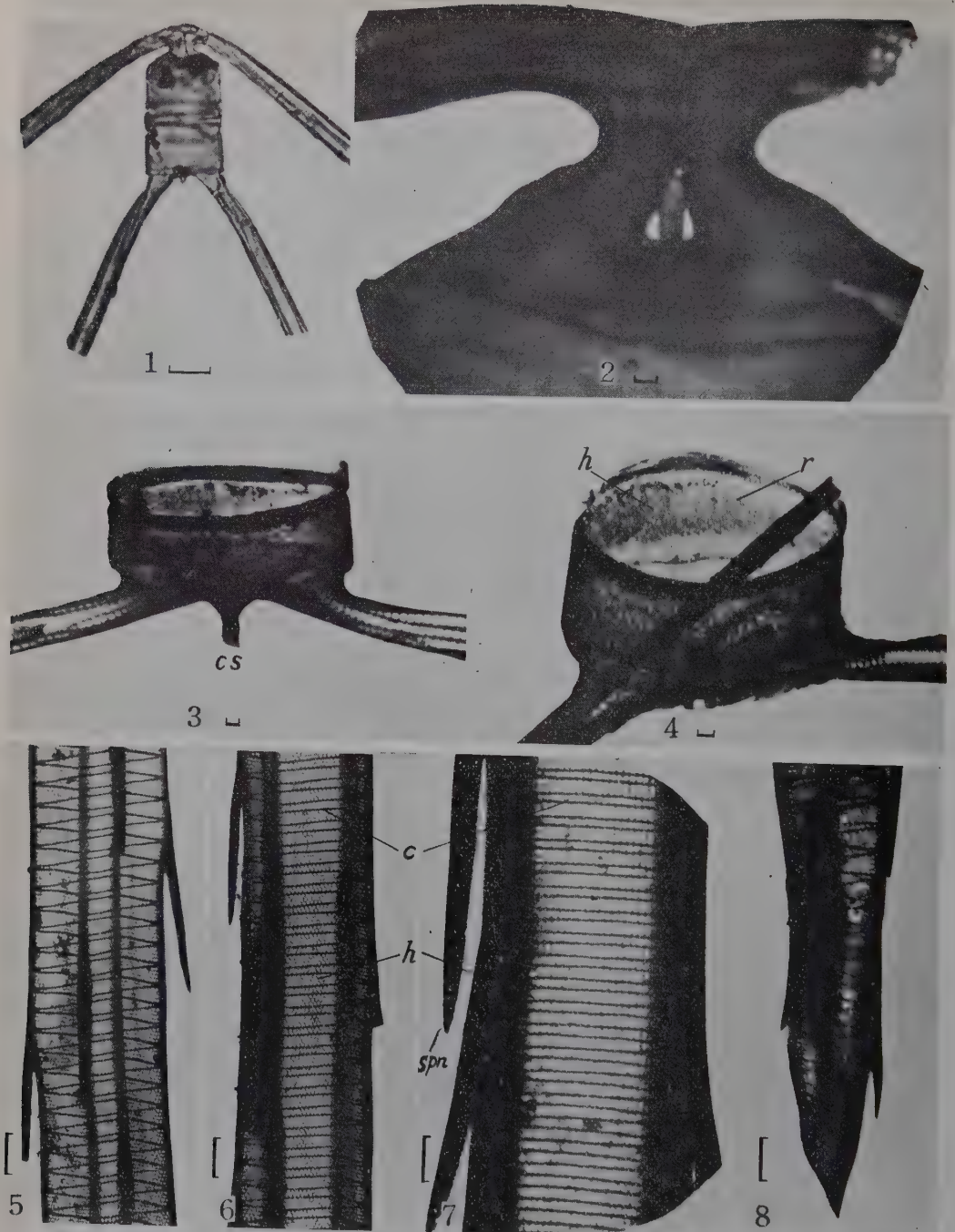
Figs. 1-8, *Chaetoceros pervianus.* 1, Single cell in girdle view. 2, Girdle view of an upper valve, showing round holes on the mantle. 3, 4, Girdle views of lower valves; the mantle in fig. 3 ribless, and in fig. 4 longitudinally ribbed. 5-8, Sections of setae viewed from various directions; in figs. 5, 6, the quadrangular prismatic structure of the wall is cleary shown. 8, Terminal portion. 1, 2. 7, 39°N; 153°E. 3, 5, 6, Akashi. 4, Hamazume. 8, Minato. (1, Light micrograph. Scale: 10μ. 2-8, Electron micrographs. Scales: 1μ.)

H. Okuno: Fine structure of diatom frustules

タバコ属植物の細胞遺伝学的研究 XIII

タバコ の 半 数 体*

竹 中　要・田 中 正 雄**

Yô TAKENAKA and Masao TANAKA: Cytogenetic studies in *Nicotiana*, XIII
Haploid plant of *Nicotiana tabacum*.

　本研究の目的は　(1) 種間交配による育種的優良形質の発見，(2) 雑種の減数分裂異常を利用しての優良劣悪両形質の分離，(3) タバコ属の核学的研究方法による系統進化の究明である。

材　　料

　N. alata の花粉に 4,800γ の X 線を照射して *N. tabacum* の 1 品種 Bright Yellow に交雑して多数の朔を得た。そのうちの 12 朔の種子約 8,730 粒を 1954 年の 2 月 10 日に温床に播種した。この交雑種子は充実不良のため発芽が悪く，僅かに約 150 箇が発芽したにすぎなかつた。しかもこの苗は非常に虚弱で，つぎつぎに枯死し，定植期には 7 本になつた。苗の全部を本圃に定植したところ，その内の 5 本は枯死し，開花期に達したものは 2 本だけであつた。その内の 1 本は茎葉が *N. alata* に酷似し，花は *N. alata* と *N. tabacum* との中間の形を具え，色は薄桃色であつた。残りの 1 本は茎葉花ともに *N. tabacum* に酷似しているが，やや小型であつた。細胞学的研究の結果は前者は種間雑種で，後者は *N. tabacum* の半数体であつた。

外　　形

　この半数体は原品種 Bright Yellow の 2 倍体と比較すると，概形がやや小型で葉が薄く，気孔の長さや細胞の大さが小さく，花粉の充実が不良で

完全に不稔である等，いわゆる半数体としての一般的特徴を示した。早蕾性ではないが，非常に早生で通常の 2 倍体よりも開花が 16 日も早かつた。また腋芽を叢生すること，柵状組織に比して海綿状組織がよく発達していること等の特徴が見られた。細胞が小さく，海綿状組織の発達が良好なことは「葉たばこ」の質が緻密で老化の進んでいることを意味するから，半数体は 2 倍体よりも品質的に良好であると推察される。また葉面における
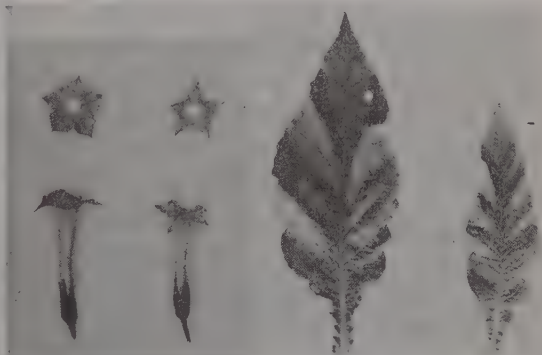
Photo.　Diploid and haploid tobaccos.
　　a.　Flowers,
　　　　left : diploid, right : haploid.
　　b.　Leaves,
　　　　left : diploid, right : haploid.

各種の毛茸については，毛茸密度と短毛歩合とは半数体がまさつているが，腺毛歩合と絨毛歩合とは 2 倍体が高い。また各種の毛茸の細胞数には両者間に大した差はないが，毛茸の長さは 2 倍体が長い。花粉充実度が圧倒的に低いのは他の半数体での通りである。(第 1 表～第 6 表参照)

第 1 表　開花期及び生育状況

| 区　分 | 開花始[1] | 草　丈 | 幹　周 | 葉　数 | 最　大　葉 | | | 腋　芽 |
					長　さ	巾	比	
X	6 月 1 日	104.0cm	7.0 cm	13.0枚	50.5 cm	18.4 cm	2.75	叢　生
2 X	6 月 17 日	161.7	8.7	17.2	53.7	19.9	2.71	少　し

1) 両者の移植期 X は 4 月 25 日，2 X は 4 月 21 日

第 2 表　葉の構造及び気孔に関する調査

区　分	全　厚	柵状組織	海綿状組織	組織肥厚比	葉長1mm当り柵状細胞	気孔密度数[2]	気孔の長さ[3]	孔辺細胞中の葉緑粒数
X	0.412 mm	0.140 mm	0.218 mm	1.60	30.9	50.5	3.24	6.53
2 X	0.450	0.172	0.212	1.26	22.4	38.4	4.42	11.10

2) 1 mm² 当り気孔数　　3) 気孔長単位 10^{-2} mm

第 3 表　毛茸密度及び各種毛茸の発生歩合

区　分	毛茸密度[4]	腺毛歩合	絨毛歩合	短毛歩合
X	17.74	72.8%	8.7	18.9
2 X	13.86	75.3	10.3	14.4

4) 葉面 10 mm² 当り総毛茸数

第 4 表　各種毛茸の細胞数

| 区　分 | 腺　毛 | | 絨毛 | 短　毛 | |
	柄	腺		柄	腺
X	3.16	1.92	3.00	1.00	5.58
2 X	3.12	2.54	3.00	1.00	6.50

第 5 表　各種毛茸の長さ

| 区　分 | 腺　毛 | | 絨毛 | 短　毛 | |
	柄	腺		柄	腺
X	21.8	4.5	10.5	3.1	3.5
2 X	29.2	6.5	22.4	3.6	3.6

第 6 表　花粉粒の充実状況

区　分	総花粉粒	充実花粉粒	不良花粉粒	充実花粉粒歩合
X	907	6	891	0.66%
2 X	1235	1195	40	96.9

減　数　分　裂

前期：染色糸の外面的変化は細糸期から太糸期まで，取立てて変つたところは見られない。しかし捻糸期から複糸期になつても染色糸の折れ曲つて，ねじれあう状態は現れない。時期の進むとともに染色糸は次第に太さをまし，移動期に入るが，染色体の対合するものはない。時おり，2～3 箇の染色体が近接して基質が接着しているではないかと思われるものがある程度である。

第一中期：ごく稀れに見られる 2 価染色体以外の 1 価染色体は，核内に分散していて，核板をつくることは殆どない。ただし 2～3 箇の染色体が二次接合的な接着，すなわち甚だしく近接していて，基質で接着していると思われるもの，或は染色体も接着しているのではないかと思われるものがある。しかしこれ等といえども核板をつくつて紡錘糸の方向に列ぶことはなく，斜や横の不規

Fig. 1.　Haploid tobacco.
MI, $2_{II}+20_{I}$.

則な型をとる。　これ等の接触集合（attached association, adherence of two chromosomes）というべきものと，ごく稀れに現われる対合（2価染色体）とを合せて，第一中期の接着型染色体の数を示すと第7表のようである。

第7表　第一中期接着型染色体数

接着型 染色体数	0	1	2	3	4	5	合計
頻度	19	38	37	17	10	3	124

これに対して核の中央にきて紡錘糸の方向に縦に列ぶ，いわゆる2価染色体と思われるものだけの頻度を見ると次のようである。

第8表　第一中期2価染色体頻度

2価染色 体数	0	1	2	3	合計
頻度	71	22	7	1	101

すなわち2価染色体のない花粉母細胞が絶対多数で，僅かに1箇の2価染色体をもつものが約22％である。2箇，3箇のものはごく稀れである。

第一後期：染色体は機会的に両極に集るが，時おり1価染色体が紡錘体の中央に残つて染色体橋をつくることがあつて染色体の断片化が起ることがある。染色体橋は1価染色体ばかりでなく，2価染色体或は接触集合型のものもつくることがある。

第二分裂：第二分裂は染色体片や紡錘体外に放出された染色体を除けば，通常の通りの分裂を行う。しかし第一分裂で両極に分れた染色体数に差のあること，或は第一分裂で3種以上に分れたもののあること，及び染色体片や紡錘体外染色体があるので，四分子期の母細胞は同形同大の四分子

ばかりを含んでいるわけではない。すなわち核の大さにも大小があり，幾分か多胞子現象が見られ，余分の微細小胞子及び微細核をもつものがある。

若い花粉：大小の小胞子は休止期に入つていくが染色糸の間に色素によく染まる球或は楕円体の染色体様のものが1～2，時には3箇も長く残つて見られることがある。稀れには染色体そのものの形を思わせるものがある。
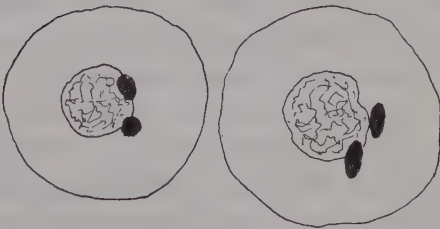
Fig. 2.　Haploid tobacco.
Two young pollen grains with chromosome-
like heteropycnotic bodies.

論　議

Clausen と Mann (1924) は *N. tabacnm* の品種 "purupurea" の半数体の減数分裂を研究して対合するものが完全にないことを報告したが，同じ材料で Chipman と Goodspeed (1927) は第一分裂中期で時おり1個の2価染色体を見た。しかし移動期や，それより前の時期に染色体の集合がないから，太糸期対合の結果による2価染色体ではなく，単に第一中期の紡錘体中で，近接した2染色体の接着のために起つたものであろうと説明した。Kostoff (1941) は *N. triplex—N. tabacum* ×(*N. sylvestris*×*N. tomentosiformis*) からできた稔性のあるタバコーと *N. sylvestris*×*N. tomentosiformis* の複2倍体との，両者からできた2種の半数体の研究をして，これ等は *N. tabacum* の半数体と同じということを見た。すなわち2価染色体の数は 0～3 箇であつて，母細胞の大多数には零であり，若干のものに1箇，それより少数に2箇で，3箇のものは極く稀れであることを見た。そして全花粉母細胞中約 27% に2価染色体があり，1母細胞当り2価染色体の数は *N. triplex* からの半数体では 0.35 であり，*N. sylvestris*×*N. tomentosiformis* の複2倍体からの半数体では 0.34 であることを報告した。Lammerts (1934)

もまた *N. tabacum* の半数体を研究して，同じく 0～3 箇の接合が起ることを見たが，温室で育てたものでは 1 母細胞当り 0.43，野外でのものは 0.18～0.19 の割合に 2 価染色体をつくつたと述べている。 Kostoff と Lammerts のものを合計して計算すると 1 母細胞当り 0.307 の 2 価染色体が見られることになるし， 1～3 箇の 2 価染色体をもつ母細胞は全体の 22% に達する。

筆者等の上記の研究においても約 30% の母細胞に 2 価染色体を見たのであるし，また 1 母細胞当り約 0.89 の 2 価染色体を生ずる計算となるから，彼等の研究と大差はないといえよう。

さて竹中 (1954, 1955, 及び印刷中) は F₁ *N. sylvestris* × *N. tomentosiformis*, F₁ *N. sylvestris* × *N. otophora* 及び F₁ *N. sylvestris* × *N. tomentosa* において，それぞれ 4 箇をモードとして 1～9 箇の， 2 箇をモードとして 0～5 個の， 3 箇をモードとして 0～7 箇の 2 価染色体を観察した。

Kostoff (1941-43) もまた F₁ *N. sylvestris* × *N. tomentosa* 及び F₁ *N. sylvestris* × *N. tomentosiformis* において，前者では 5 箇以上は非常に稀れであるが 1～4 箇の 2 価染色体を見ているし，後者でもほぼ同様の 2 価染色体が形成されることを報告した。 Goodspeed (1934, 1954) もまた tomentosa group の *N. otophora*, *N. Setchellii* 及び *N. tomentosa* と *N. sylvestris* との交配 F₁ において， 3 者とも 3 箇をモードとして 0～7 箇の 2 価染色体が形成されるのを見た。 すなわち Kostoff, Goodspeed 及び筆者の研究はほぼ一致する。

N. sylvestris と Tomentosa group のあるものとのゲノムが *N. tabacum* の 2 つのサブゲノムであるということは，ゲノム分析からも，また *N. tabacum* の合成からも既に証明されている。しか

るに *N. tabacum* の半数体では対合する 2 価染色体の数が甚だ少なく，これに反し *N. sylvestris* と tomentosa group のものとの F₁ ではそれが多数見られるのは何故であろうか。

N. tabacum の 2 つのサブゲノムは天然にタバコが合成された最初は高い二重性の完全な構成を持つていたに違いないが，長い間にその二重性を減じ 2 つのサブゲノムの間の雑種相同性 (cross homology) が減少したのであろう。 (Clausen 1941)

事実種々の変異形質をもつ *N. tabacum* の品種間の交配の研究においても同様の現象が見られる。すなわちこれは交配による形質の研究と，染色体の対合との間に平行関係の見られる好例ということができよう。

なおここに一層面白いのは，第 7 表に示した通り，接合染色体 (2 価染色体) と接触集合型のものとを合せた全接着染色体が， 1～2 をモードとして 0～5 箇存在することである。 これは tomentosa group のものと *N. sylvestris* との交配 F₁ の 2 価染色体の数に近似である。 このことは *N. tabacum* が天然に合成されて以来長年の間に雑種相同性の減少が起つていても，なおかつ 2 つのサブゲノムの若干数の染色体の間に，相同の遺伝子が存在していて，接合に到らないまでも，少量の親和性の名残をとどめて，接触集合を起したものと考えることができよう。

ただここに一つ不思議であるのは， Kostoff のつくつた *N. triplex* と *N. sylvestris* × *N. tomentosiformis* の複 2 倍体とからの半数体に於て， 2 価染色体が余りにも少ないことである。この両者は未だ十分老熟した親からきた半数体とはいえないから，筆者等は例外的事実としてしか取扱うことができない。

Abstract

A variety of *Nicotiana tabacum*, "Bright Yellow" was pollinated with pollen of *N. alata* treated by X-rays (4,800 γ). From the seed obtained, 8,730 were sown, 150 germinated but only two plants reached maturity. One of them was a hybrid, and the other was a haploid tobacco plant which was a little smaller than the parental diploid "Bright Yellow."

In studying MI in PMC's of the haploid plant, most frequently 24 univalents

were found; in about 30 % of PMC's one to three bivalents were observed. On the average 0.39 bivalents occurred per PMC.

An early study on meiotic behavior in haploid *N. tabacum* "purpurea" was reported by Clausen and Mann (1924). They indicated complete lack of pairing at MI, while in the same material Chipman and Goodspeed (1927) saw an occasional bivalent and interpreted it as a product of adherence of two chromosomes closely associated on the MI spindle rather than a reflection of pachytene pairing, since association was not observed at diakinesis or earlier. Kostoff found in a haplont of *N.* "*triplex*"—*N. tabacum* × (*N. sylvestris* × *N. tomentosiformis*)—and in a haplont of amphidiploid *N. sylvestris* × *N. tomentosiformis*, both of which he considered equivalent to haploid *N. tabacum*, a range of zero to three pairs. The former had 0.35 and the latter 0.34 bivalents per PMC. The above mentioned observations in the haploid tobacco plant agree with these results.

On the cotrary, in the three hybrids, *N. otophora* × *N. sylvestris*, *N. sylvestris* × *N. Setchellii*, and *N. sylvestris* × *N. tomentosa*, Goodspeed (1934, 1954) found a range of zero to seven bivalents with the mode at 2–3, and also Takenaka (1954, 1955) counted, in the hybrids *N. sylvestris* × *N. tomentosiformis*, *N. sylvestris* × *N. tomentosa* and *N. sylvestris* × *N. otophora*, 1–9 bivalents with the mode at 4, 0–7 with the mode at 3 and 0—5 with the mode at 2 respectively, in the above cited order. Takenaka's observations generally agree with Goodspeed's findings concerning chromosome affinity between the sylvestris genome and those of the tomentosa group.

Accordingly, bivalent number at MI of the hybrid between *N. sylvestris* and the species of tomentosa group is always higher than that of haploid tobacco. Concerning the decrease of intragenomatic affinity in haploid tobacco, Clausen (1941) advocated the following assumption: "the alterations which have diminished its duplicational completeness must have arisen largely since it became established as an amphidiploid."

On the contrary, in the hybrid *N. sylvestris* × *N. tomentosa*, Kostoff found usually univalent chromosomes or 1 to 4 bivalents, rarely more than 4, and in F_1 *N. sylvestris* × *N. tomentosiformis* somewhat less bivalents than in the former. He stated that meiosis of the *N.* "*triplex*" haploid and the haploid of amphidiploid *N. sylvestris* × *N. tomentosiformis* could not be distinguished from the meiosis in F_1 *N. sylvestris* × *N. tomentosiformis*.

The difference between Kostoff's and Goodspeed's as well as Takenaka's findings in the hybrids between *N. sylvestris* and tomentosa group are ascribed to the different methods of cultivation. Nevertheless, it is certain that the bivalent number in haploid tobacco is less than that of the hybrids between *N. sylvestris* and species of the tomentosa group.

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花 粉 の 生 理 學 的 研 究　X

糖の吸收と 2, 3 の酵素作用について*

岩 波 洋 造**

Yōzō IWANAMI: Physiological Researches of Pollen X
The Absorption of Sugars and some Enzyme Reactions.

前報[1] では花粉が形成される過程において幾度か細胞内澱粉粒の消長がみられること, 及び開花時の花粉は sucrose, glucose, fructose などの糖をもつていることなどについて述べたが, 今回はこれらの花粉が花粉管を伸して生長する間に外から吸收する糖の種類, 及び amylase など炭水化物分解酵素についての実験の結果を報告する。

I　培養基からの糖の吸收

花粉が生長して伸す花粉管の長さは, 自体の幾千倍にも達するのであるから, この間において管膜の材料に使用されたり, 呼吸の為に消失する炭水化物の量は, 開花時に花粉が自已の中に貯えている僅かの種類で間に合う筈がない。それにもかゝわらず従来花粉が外からの養分を使つていることについての結論がでていないのは, 人工培養に際して必ずこの中に sucrose などの糖を含ませてはいるが, これらの糖が花粉の破裂を防ぐ為に外液の滲透圧を調節する役目を一方で果していることがあまりにも明らかであつたからであろう。又花粉が外からこれらの糖を吸收することが実験的に認められていなかつたこと, 更に或る種の花粉は無糖の培養基でも可成り花粉管を伸すことなどがこの辺の問題を複雑にしていたものと考えられる。Fig. 1 は寒天 1.5% pH 6.5 の無糖の培養
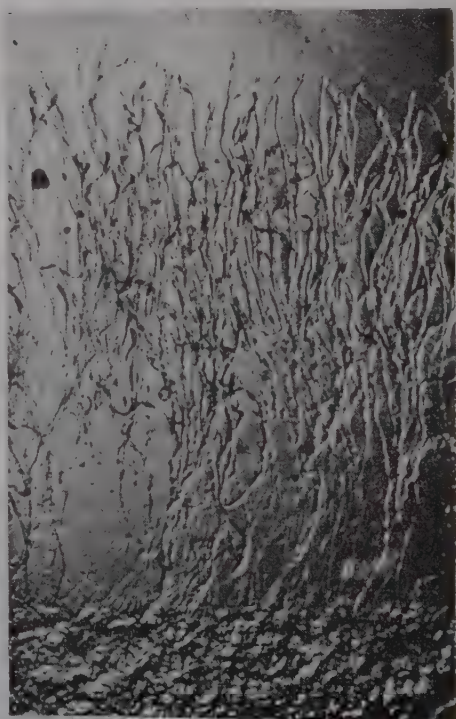
Fig. 1.　無糖の培養基（寒天 1.5%）上で伸長する *Impatiens Balsamina* L.　の花粉管

** 横浜市立大学文理学部生物学教室

基上で花粉管を伸した *Impatiens Balsamina* L. の花粉である。このような特殊なものを除いては多くの花粉は無糖の寒天のみの培養基では破裂したり，不発芽に終つたりするし，更に糖の中でも sucrose を加えることが他のものを加えるよりも常に好結果を得ることができるところから，従来 sucrose を用いるのを常としているようである。

とも角花粉を人工培養するに際して，外から何かを内にとり入れたとすれば，培養基からその分が消失することになる。そこで培養基中に種々の糖をあらかじめ含めて花粉を培養し，培養基内の糖の消失をみれば，何を花粉が吸收したかがわかる。これについては最近志佐氏[2]らがペチュニヤに関する一連の実験の中で，ペチュニヤの花粉を長時間培養した培養基から sucrose と僅かの glucose が消失したと報告している。

実　験　1

前述のごとく *Impatiens Balsamina* L. の花粉と，*Lilium longiflorum* Thunb. が，一方は glucose を主として持ち，他方は sucrose を主としてもつ点で対照的な花粉と考え，更に *Impatiens* の花粉は無糖の培養基上でも可成りの花粉管を伸すことをも考慮に入れて，この2種類の花粉について糖の吸收を調べた。

培養基に含めた糖の種類は，花粉の中に検出された[1] sucrose, glucose, fructose の3種類と，澱粉の分解過程における生成物の一種であると考えられる maltose の4種類を使用した。

方法は sucrose 0.1 g, fructose 0.1 g, glucose 0.05g, maltose 0.1g を 8cc の蒸溜水（pH 6.5）に溶かし，これに寒天 0.12 g を加えて寒天培養基を作つた。これを厚さ 0.7 mm, 長さ 20 mm, 幅 10 mm の板に切つてスライドグラスにとり，同様のものを 10 個用意した。

培養基に含める糖の濃度や寒天板の大きさは，あらかじめ幾度か行つた予備実験の結果から最も適当と思われるものを選んだ。例えば glucose を他の糖の半量としたのは後に行うクロマトグラフの実験で，4者の糖がほゞ等大のクロマトグラムを形成する目的を有している。

10 個の寒天培養基の中で5個は *Impatiens* の為に，他の5個は *Lilium* の花粉の為に使われ，この中両者とも1つづゝは比較の為にそのまゝ残

し，他の4つづゝにそれぞれ花粉を表裏に約2mm 間かくに格子状に散布した。即ち全面に密に花粉をまかずに花粉管の伸びる場所を与えるように散布した。（Fig. 1 参照）

これを 27℃ の湿室に納めてから1時間，2時間，3時間（*Lilium* は4時間），20時間の各時間を経過した時に1つづゝとつて花粉をとり除いた後，寒天板を 2cc の蒸溜水の中ですりつぶした。よくかくはんした後に上清液を 0.06cc づつガラスの細管でとつて濾紙に与え，これをペーパークロマトグラフの実験の系列（前報同様）に移した。
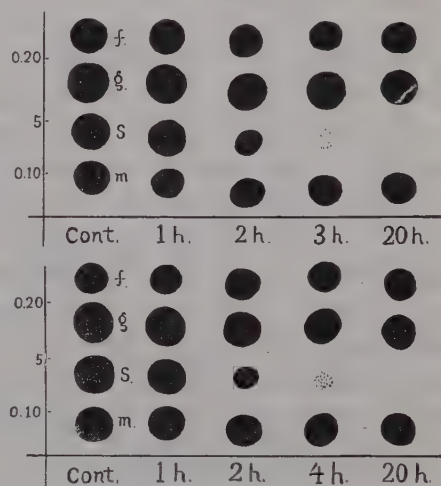
Fig. 2.　培養基からの糖の消失（sucrose が消えている）
上段—*Impatiens Balsamina* L.
下段—*Lilium longiflorum* Thunb.

18時間展開後に得られたクロマトグラムが Fig. 2 に示されている。これによつて，量的な差は問題にできないが *Impatiens*, 及び *Lilium* の花粉の何れもが培養基から sucrose のみを吸收したと考えることができる。

なお sucrose がない時には他の糖をも吸收するかもしれないとの考えの下に，この実験と同時に glucose, fructose, maltose の3種類，及び glucose, fructose の2種類を含む培養基での糖の吸收を調べたが，24時間後も sucrose 以外の糖は殆ど吸收される様子がみられなかつた。また *Camellia japonica* L. の花粉を用いた時は，花粉管が十分伸長しているにもかゝわらず吸收される糖の種類を確かめることができなかつた。これらの

ことは花粉の種類によつて糖の吸収の仕方を異にすることを示唆するものと思われる。即ち或るものは外部から大量に糖をとり入れて内に貯えながら生長を行うし，或るものは必要に応じて糖を吸収するというような違いがありはしないかと筆者は考えている。上述の *Lilium, Impatiens* などの花粉は花粉管が可成り伸長してからも澱粉粒を保有しているに対し（この澱粉粒は培養基にまかれて後に再形成されたものである），*Camellia* の花粉では，花粉管中に澱粉粒がみられないことなどはこの辺の事情を物語つていると考えられる。

ともかく花粉は生長を行うに際して sucrose の形で炭水化物をとり入れていると考えてよいであろう。このことは従来，人工培養基に sucrose を加えると最も花粉の発芽に好結果が得られていることを是認するし，*Lilium* の花粉が開花時に sucrose を貯えていること，及び別の実験（未発表[3]）で蕾の柱頭には澱粉粒が多く含まれているが，花の時期にはこれを sucrose などの糖に変えていることとも矛盾しないと考えられる。ただ glucose を主として保有する *Impatiens* の花粉が *Lilium* 同様 sucrose を吸収したことは，生体内における糖の転移の問題に関連して注目すべきことであろう。

II　澱粉及び糖の分解作用

既に花粉が開花の時期になるにしたがつて澱粉粒を消失することを報じたが，このことは花粉の中に amylase の存在することを示唆する。これに関しては古く Paton[4] らが花粉を可溶性澱粉液に入れて長時間おくと還元糖がそこに生成されることを Fehling 液の反応で確かめている。筆者は更に花粉に amylase が存在することを確認するために，生成される糖の種類をペーパークロマトグラフで調査した。

実　験　2

0.1 g の可溶性澱粉を 15 cc の蒸溜水（pH 6.5）に溶かし，これを 2 cc づつ蓋付きのガラス製小びんにとつた。この中に 0.05 g の *Lilium longiflorum* Thunb. の花粉を入れてすりつぶし，遠心分離器にかけて沈澱物をとり除いた後に 28°C の湿室に納めて時間の経過と共にヨード反応及びクロマトグラフによつて糖の生成を調査した。

用いた花粉は花の時期の花粉（XV），蕾の時期の花粉（XIII），及び lactose 10% の培養基で 60 分培養後の花粉の 3 種類である。この中ヨードの反応は花の時期の花粉についてのみ調べた。その方法は 20 cc の中から 3 cc づつ時間の経過と共に蒸発皿にとつてヨード・ヨードカリ液を加えて色調の変化を調べたが，なおこの変化が酵素作用によるものであることを確かめる為に，同様のものを 2 個用意し，その中 1 個は花粉の汁液を加えてからさらに一度煮沸して色調の変化を比較した。

結果は Fig. 3 及び表 1 に示されている。
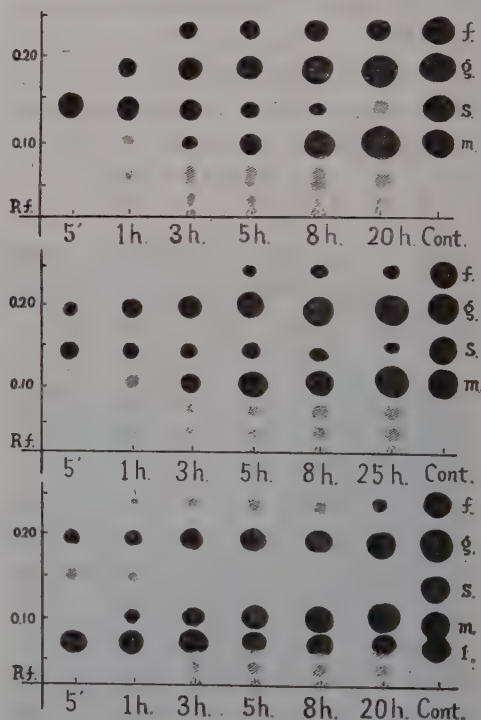
Fig. 3.　可溶性澱粉液に花粉の汁液を加えた時の糖の生成
上段—開花時の花粉　中段—蕾の花粉
下段—培養中の花粉
（*Lilium longiflorum* Thunb.）

表 1　花粉の汁液を加えた可溶性
　　　澱粉液の色調の変化

時　　　間	0	1	3	5	20
花粉汁液を加えた可溶性澱粉液	青	青紫	紫	紫褐	黄褐
同上（煮沸）	青	青	青	青	青

Fig. 3 のクロマトグラムでわかる通り，可溶性澱粉液内には，次第に maltose と glucose 及び 2, 3 の未詳の糖が生成され，一方ヨードの反応は青色から薄黄色に変つている。またこの反応は一度煮沸したものに於ては最後まで澱粉の反応である青色を示していることなどから，花粉の中に amylase が存在していると認めてよいと思われる。

なお Fig. 3 で最初から glucose 又は sucrose がみられ，又 fructose が生成されているのは，次の実験で明らかなように，花粉の中に含まれていた sucrose が同時に分解された結果であろう。

実　験　3

実験 2 で sucrose の分解のこん跡がみられ，また Lilium longiflorum Thunb. の花粉が前述[1]のごとく sucrose を多く含んでいながら培養基に移されると次第に glucose を多くもつようになることから，花粉の中に invertase が存在することを推察し得る。これに関しても古く Paton[4]らが種々の花粉を砂糖液中に入れて長時間放置すると還元糖が作られることを Fehling 液でみている。

筆者は澱粉の分解作用と同様の方法で sucrose が分解されて後にそこに形成されてくる糖の種類を調査した。

sucrose 0.1 g を 8 cc の蒸溜水 (pH 6.5) に溶かして 2 cc づつとり，この中に Lilium longiflorum Thunb. の花粉 (sucrose を自己の中にもち，sucrose を外から吸収する) と Impatiens Balsamina L. の花粉 (sucrose を外から吸収するが，自己の中には glucose を保有している) を別々に入れてすりつぶし，遠心分離器にかけて固形物を除いたものを 28°C の湿室に納めた。

これらについて時間の経過と共に前記同様の方法でクロマトグラフの実験の系列に移して糖の変化を調べた結果が Fig. 4 に示されている。

即ち Lilium の花粉も，Impatiens の花粉も共に sucrose を glucose と fructose に分解した。一度煮沸したものでは最後まで sucrose がそのまゝ残つていることから，これらの花粉の中に invertase が存在すると考えてよいと思われる。

同上の方法で sucrose, glucose, fructose の 3 種類を同時に加えた液に花粉汁を加えた場合には

sucrose だけが消えて glucose, fructose がふえ，glucose, fructose, 及び maltose, lactose などを各単独に溶かした液については，何れも他の糖に変化するのがみとめられなかつた。これらのクロマトグラムについてはここでは省略するが，Hibiscus mutabilis L. の蕾の花粉について調べた時に invertase の作用と共に fructose を単独に与えた時にこれを glucose に変える作用のみられたことを附言しておく。
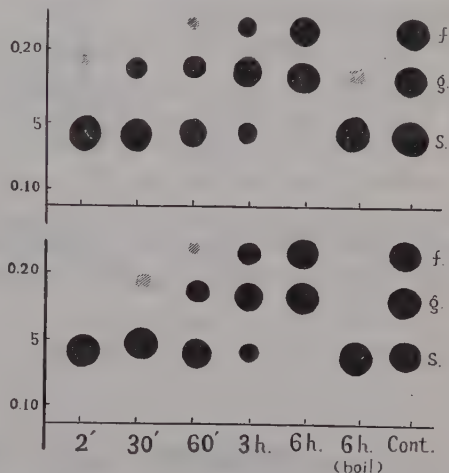
Fig. 4. 花粉の汁液を加えた時の sucrose の分解
　　　上段—Impatiens Balsamina L.
　　　下段—Lilium longiflorum Thunb.

とも角花粉の中に amylase, invertase があることは明らかであるが，たゞここで問題となる点は第 1 に花粉が蕾の時期から開花の時期において澱粉粒を消失する時，前述[1]のごとく maltose の存在が全く認められないことである。また maltose の液に花粉の汁液を加えた時も maltose を分解する作用が認められなかつた。第 2 の点は sucrose を次第に失つて glucose を主としてもつようになる Impatiens の花粉が invertase をもつことは当然であるが，逆に glucose を失つて sucrose をもつようになる Lilium の花粉の中にも強力な invertase の存在がみられた。これら生体内における特有な酵素作用の進行について今後更に追究してゆくつもりである。なお phosphorylase などの作用については，項を改めて報告する。

実験に協力された富永敦子君に感謝の意を表する。

Summary

1)　The absorption of sugars from artificial culture media and enzymes reactions in the pollen were investigated with paper chromatographic method.

2)　The pollen of *Lilium* and *Impatiens* absorbed only sucrose from the culture media throughout their growth (Fig. 1).

3)　By addition of pollen juice of *Lilium longiflorum* Thunb. to the solution of soluble starch, glucose, maltose and some other unknown sugars were regularly produced, while the color of iodine reaction of this solution turned into yellow brown from blue (Fig. 4).

4)　By addition of pollen juice of *Lilium* and *Impatiens* to sucrose solution, glucose and fructose were produced, then sucrose was regularly removed (Fig. 4). It may be said that these pollens have some amylase and invertase.

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遊離葉緑体における澱粉の形成について　II

小　野　　林[*] ・ 小長光与壯[*]

Hayashi ONO and Yoso KONAGAMITSU: The Formation of Starch
in the Isolated Chloroplasts II.

著者等は別報[14] で chloroplast, cytoplasm 何れにも phosphorylase が存在し, その作用は温度や光等の外的要因の変化によつて起る細胞内の生理的条件の変化によつてかわる事を明らかにした。即ち高温・明条件におかれた葉の細胞より遊離された chloroplast や cytoplasm は低温・暗所におかれた葉の場合よりも starch の形成作用が著しく大である。 しかも それ等要因の影響は chloroplast や cytoplasm を細胞から遊離させる前に行われると, 遊離させた後に同様に処理したものよりも phosphorylase の作用に一層効果

的である。 他方著者[11,12] は細胞内の phosphorylase や amylase の作用は細胞内の水素イオン濃度の変化に支配され, その変化によつて phosphorylase と amylase の作用の相互転換が起る事を明かにした。しかし此等の現象の原因やその機構の説明はまだ不充分である。著者等は此等変化の機構や, 酵素作用と温度や光条件の影響の関係を明かにする為に細胞より遊離された chloroplast や cytoplasm の starch 形成と, phosphorylase の作用に特に影響すると思われる amylase や phosphatase の作用をそれ等酵素阻害剤である HgCl$_2$ や NaF を使用して追求した。此の 実験 より 細胞内に おける phosphorylase, amylase, phosphatase の作用関係を知る事が出来たのでその結果をかんたんに報告する。

* 九州大学教養部生物学教室 (Biological Laboratory, General Education Department, Kyushu University).

実験材料と方法

前報[14]におけると同様に *Bryophyllum, Raphanus* の葉を主として用い，その他 *Trifolium, Plantago, Solanum* や *Erigeron* の葉を用いた。酵素阻害剤として，10^{-3}～10^{-5} Mol. の NaF と $HgCl_2$ を使用した。$HgCl_2$ の 10^{-3} Mol. 以上の高濃度は phosphorylase の作用を阻害する。又 0.01% の 2,4-dinitrophenol や rhodamin B 添加の影響も見た。実験に使用した α-amylase は Meyer (1948) の方法により唾液より調製し，β-amylase は Balls (1948) の方法により甘藷から調製した。その他の方法は前報[14]におけると同様である。

実験結果と考察

1. $HgCl_2$ と NaF 添加の影響: 2-5 日間暗所におかれた大根葉から遊離された chloroplast や cytoplasm では G-1-P を基質として starch が形成される事があるが phosphorylase の作用は極めて弱い。然し此の場合基質に $HgCl_2$, NaF を添加すると starch の形成は促進され，特に chloroplast よりも cytoplasm において著しい (Table 1)。前報で明かにした様に光条件におかれた葉より遊離されたものは暗条件におかれたものより starch がよく形成されるが，此の際 $HgCl_2$, NaF の添加で starch の形成は尚一層促進される。これは phosphorylase そのものの作用力が光条件において促進された事を示す。

一般に $HgCl_2$ は amylase (Nakamura, 1952)，NaF は phosphatase (Porter, 1953, etc.) の選択的阻害剤として知られている。それで此等の阻害剤の添加は phosphorylase 作用を害する amylase や phosphatase の作用を阻害し，その結果 phosphorylase の作用のみが現れ，chloroplast や cytoplasm 中の starch 形成が促進される。

上述の事実は細胞内に於て温度や光の変化の影響で変化する水素イオン濃度によつて主として作用する酵素系の作用転換が起るという著者[9,11,12] の考を証明するものである。即ち葉肉組織細胞内の水素イオン濃度は露光により大となり，その結果 phosphorylase の作用は大となるが amylase の作用は小となる。暗所では逆に細胞内の水素イオン濃度は小となり phosphorylase の作用は小となり amylase の作用は大となる。此の理由から暗処理されたものよりも，光条件におかれた細胞から遊離された chloroplast や cytoplasm に phosphorylase の活性度が大であり starch 形成も多く，又 $HgCl_2$ や NaF の添加で phosphorylase に拮抗的に作用する amylase, phosphatase の作用を除く為に phosphorylase の作用がなお強く現れるのである。暗所におかれたものも amylase の作用に阻害されて，そのまゝでは phosphorylase の作用が現れないが，阻害剤の添加で phosphorylase の作用のみが現れる為に starch が形成される。

他方 NaF の添加で starch の形成が促進される理由に関して，これは phosphorylase の phosphatase による直接の阻害作用を除く理由の外に phosphatase 作用の除去により燐酸エステルの蓄積が起り，水素イオン濃度大なる条件において Free P/G-1-P の平衡を大に保つ為に著者が先に報告[11,12]した様に starch の形成が起るのである。

$HgCl_2$ と NaF は *in vitro* において phosphorylase の作用や，starch 形成を促進しないばかりでなく，濃度が高いと反つて阻害作用が現れるが，*in vivo* や，遊離された chloroplast や cytoplasm においてこれ等の添加で starch 形成が促

Table 1.　The effect of $HgCl_2$ and NaF on the formation of starch in the isolated chloroplasts and cytoplasm from the leaves of *Raphanus sativus* L.

	In dark		In light	
	Chloroplasts	Cytoplasm	Chloroplasts	Cytoplasm
Control	±	+	+	+ ～ ╫
$HgCl_2$	+	+ ～ ╫	╫ ～ ╫╫	╫╫
NaF	+	+ ～ ╫	╫ ～ ╫╫	╫╫

Table 2.　The effect of HgCl2 and NaF on the formation of starch in isolated chloroplasts and cytoplasm from the leaves of various plants kept in the dark three days.

Materials	Parts	Control	HgCl2	NaF
Trifolium repens L.	Chloroplasts	− ～ ±	+	±
	Cytoplasm	+	‖	‖
Plantago major L.	Chloroplasts	− ～ ±	+	± ～ +
	Cytoplasm	+	‖	‖
Solanum tuberosum L.	Chloroplasts	− ～ ±	± ～ +	± ～ +
	Cytoplasm	‖	‖	‖
Erigeron annuus L.	Chloroplasts	− ～ ±	± ～ +	± ～ +
	Cytoplasm	+	‖	‖

Table 3.　The effects of 2, 4-dinitrophenol and rhodamin B on the formation of starch in the chloroplasts and cytoplasm isolated from the leaves of *Raphanus sativus* L. that kept in the dark 2-5 days.

	Chloroplasts	Cytoplasm
Control	±	+
2, 4-Dinitrophenol	+	+
Rhodamin B	+ ～ ‖	‖ ～ ‖

Table 4.　The effect of amylase on the formation of starch in the chloroplasts and cytoplasm isolated from the leaves.

Materials	*Raphanus sativus* L.				*Bryophyllum calycinum* S.	
Treatment	Dark		Light		Dark	
Parts	Chloroplasts	Cytoplasm	Chloroplasts	Cytoplasm	Chloroplasts	Cytoplasm
Control (G-1-P)	±	+ ～ ‖	+	‖	‖	+
G-1-P+α-amylase	±	−	+	±	+ ～ ‖	±
G-1-P+β-amylase	−	−	+	±	+	−

進されるのは，之等の物質が直接 phosphorylase の作用を促進するものでなく，間接的な作用である。即ち細胞内で phosphorylase の作用に直接，間接に影響する酵素作用系の破壊によるものである。Table 1 及び 2 で明白な様に HgCl2 や NaF の添加で starch の形成は chloroplast よりも cytoplasm において著しく促進される。これは cytoplasm では此等の物質が直接 amylase や phosphatase と接触し作用を阻害するが，chloroplast ではそれを構成する膜が此等の阻害剤の透入を阻げる為 amylase や phosphatase に及ぼす阻害作用が cytoplasm の時の様に強くないためと思われる。又前報[14]で報告した様に大根葉では chloroplast よりも cytoplasm 中において phosphorylase の活性度が大なる為である。

　2.　2, 4-dinitrophenol と rhodamin B 添加の

影響: Table 3 に示す様に之等の物質の添加は遊離された chloroplast や cytoplasm の starch 形成を或程度促進し特に rhodamin B 添加の効果が著しい。又 chloroplast よりも cytoplasm においてよく促進される。rhodamin B の細胞内 chloroplast の starch 形成促進作用については，植田[19] により報告されているが，細胞から遊離された chloroplast においても同様である。

3. amylase 添加の影響: 孔辺細胞の starch 形成が β-amylase の添加で阻害され，又 in vitro では α- 又は β-amylase の添加で phosphorylase 作用は完全に阻害される事について著者[10]は先に報告した。細胞より遊離された chloroplast や, cytoplasm では Table 4 に示した様に α-, β-amylase の添加で starch 形成は著しく阻害された。即ち光条件の葉より遊離された chloroplast や cytoplasm の phosphorylase 作用は暗処理のものよりも強いが，此の際 amylase の添加で phosphorylase の starch 形成作用は阻害され，特に cytoplasm において著しい。又 β-amylase の添加で著しく阻害された。HgCl$_2$ や NaF の添加で cytoplasm の starch 形成が chloroplast よりも促進されたと逆に amylase の添加で cytoplasm における phosphorylase の作用が著しく阻害される。此の様に chloroplast と cytoplasm の phosphorylase の作用が amylase の添加で異るのは前述と同様に chloroplast の膜の存在による透入の困難の為である。α-amylase の阻害作用が chloroplast よりも cytoplasm に強い事は α-amylase が β-amylase におけると同様に細胞内において phosphorylase の作用に重要な生理的意味を持っていることを示す。amylase を添加する前に chloroplast 中に形成された starch はその後に amylase を添加しても消失しない。

結　論

細胞内において starch と sugar の相互転換に関係する phosphorylase, amylase や phosphatase の作用関係は一定の条件において生理的に調整され，平衡を得ている。然し外的条件の温度・光やその他内的生理的要因の変化は此の様な酵素作用系の平衡を乱す。ある平衡した条件において細胞を破壊し chloroplast や cytoplasm を細胞から遊離させ，HgCl$_2$ や NaF の amylase や phosphatase の酵素阻害剤を加える事によって phosphorylase の作用を比較的強く現す事が出来る。暗処理し細胞内の amylase 作用を強めて phosphorylase の作用が現れない状態のものも HgCl$_2$ を加え amylase 作用を除く事によって遊離された chloroplast や cytoplasm に phosphorylase の starch 形成作用を現出させる事が出来る。NaF の添加は細胞より遊離された chloroplast や cytoplasm の phosphatase の作用を阻害し phosphorylase の starch 形成作用は促進されるが，その理由の一つは，phosphorylase の作用を阻害する phosphatase の作用を除く直接的な効果と，他方 phosphatase 作用の阻害によって燐酸エステルの蓄積を来し一定の水素イオン濃度における free P/G-1-P の平衡を維持する為に starch の形成が促進されるものと考えられる。又反対に α- や β-amylase の添加によって細胞より遊離された chloroplast や cytoplasm の starch 形成は著しく阻害される。

starch 形成や分解に関与する之等の酵素作用間には拮抗作用的関係があり，主として作用する酵素の種類や作用力が光や温度の外的環境要因の変化，及びそれ等の変化より誘導される内的生理的要因の変化，特に水素イオン濃度の変化で決定されるという事実が，これ等酵素の作用関係を見る事により明かである。

Summary

1. The formation of starch in the isolated chloroplasts and cytoplasm was accerelated by the addition of HgCl$_2$ that inhibits the action of amylase.

2. The addition of NaF that inhibits the action of phosphatase also accerelates the formation of starch in the isolated chloroplasts and cytoplasm.

3. These reagents are effective in concentrations of 10^{-3}—10^{-5} Mol. as

inhibitors.

　　4. The addition of these reagents does not accerelate the starch forming action of phosphorylase *in vitro*, but the elimination of amylase and phosphatase activity by the treatment correlatively and indirectly accerelates the action of phosphorylase.

　　5.　The addition of rhodamin B and 2, 4-dinitrophenol accerelates the formation of starch in the isolated chloroplasts and cytoplasm.

　　6.　The addition of amylase inhibits the formation of starch in the isolated chloroplasts and especially in cytoplasm and in this case the inhibitory action is stronger in β-amylase than in α-amylase.

　　7.　There exists the antagonistic relation concerning the action of phosphorylase, amylase and phosphatase, and this relation is controlled by the change of hydrogen ion concentration in the cell.

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日本学術会議会員選挙について

日本学術会議中央選挙管理会より日本学術会議第四期会員選挙の実施に関する下記事項について会員各位に周知方を依頼してきましたので，お知らせします。

　　　　　　　　記

1.　選挙権・被選挙権をもつためには

本年 12 月 10 日に日本学術会議第四期会員の選挙が行われるが，選挙権を行使し又は選挙されるためには，登録用カードを提出し，本管理会で認定されなければならない。

2.　登録用カード用紙について

(1)　当管理会からは，

　(イ)　前回（昭和 28 年）の有権者名簿に登録した者のうち，当時，大学・研究機関に勤務していた者に対しては，その勤務先を通して登録用カード用紙を送付する。

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　　　　　　　〔葉　　　書〕

[註] 葉書を縦にして横書のこと。

登録用カード用紙請求書

氏　名（必ずふりがなを付すこと）

現住所

勤務先・職名

3.　登　録　期　間

登録用カードを提出する期間は，5 月 1 日から 7 月 20 日までである。

- -

役員の・移動

編集委員　門司正三氏が辞任し，次の 3 氏が新しく会長から指名された。

　　前川　文夫　　高宮　篤　　原　　寛

幹　事　4 月から一部の幹事が交代し，次のようになつた。

　　庶務幹事　　原　　襄
　　会計幹事　　代谷　次夫
　　編集幹事　　駒嶺　穆
　　　　　　　　西村　光雄
　　　　　　　　倉石　晋
　　図書幹事　　古沢　潔夫（生物学抄録）
　　　　　　　　金井　弘夫

支　部　通　信

関東支部

3 月例会（3 月 17 日，於東大農）薬師寺英次郎，松崎悦三：緑色植物のポリフェノラーゼに関する研究．高橋基生，他 5 名：イネ科植物の根茎呼吸の微量測定及び之が土壤並びに培養液との関係より植物の分布並びに新栽培法に及ぶ。

九州支部

第 38 回例会（2 月 4 日，於九大理）中村和郎：アカパンカビにおける子嚢胞子着色因子について．服部静夫：ゴマノハグサ科，シソ科の糖類について。

投稿の注意

1)　投稿は会員に限ります。

2)　原稿は簡潔に書き，原則として刷上り 6 ページ（図表，摘要，文献を含む）以内で，他に印刷されていない内容であること。

3)　欧文（英，独，仏，ラテン）原稿は，必ず片面に 1 行あきに黒リボンを用いタイプライト印書すること。用紙は標準型（約 30×21cm 又は A 4 版），薄紙をさけ，上下左右を 3-4cm あけてうつこと。Elite (small) タイプを用いた時は 2 行あき。コピーでなく正本を送ること。

4)　和文原稿は 400 字詰原稿用紙に平がなで横書きにし，現代かなづかいを使うこと。学術用語はなるべく，文部省編，学術用語集植物学篇（1956 年 4 月発行）に従うこと。なお刷上り 1/2 ページ以内の欧文摘要をつけること。

5)　表題及び著者勤務先は和欧両文で書くこと。

6)　図や表はできるだけ小さくし，本文とは別紙に書くこと。図の説明も図とは別に書き，本文中に図を入れる位置を明示してそこに説明をはつておくこと。図は刷上りでページ幅か又は横に 4cm 以上のあきが必要。図や文字の大さ・太さは希望する刷上りの大きさの 3/2 倍又は 2 倍位に書くのが適当。凸版の図中に入れる文字は，印刷した活字をはり込むか，黒インクでていねいに書くこと（タイプライトしたものは不可）。図の裏には著者名，表題，図の番号を書いておくこと。

7)　文献引用の形式その他の記号は，本誌最近号を参照のこと。

（例）　Bot. Mag. Tokyo **69**: 192 (1956)

Journ. Fac. Sci. Univ. Tokyo III, **6** (1): 1 (1954)

8)　速報は緊急発表を要するものに限り，刷上り，1 ページ以内のものとし，1 ページ 1500 円をそえて投稿のこと。

9)　原稿の採否，修正，体裁及び登載の順序はすべて編集委員会の決定によります。特に編集委員会が認めた場合は，制限ページ数を越えた分及びアート図版を著者の実費負担で載せることがあります。

10)　活字指定は編集幹事がしますから，著者が特に希望する個所があれば黒鉛筆で指定しておくこと。

11)　校正は原則として編集幹事に一任のこと。

12)　別刷は 50 部を進呈します。それ以上を著者の実費負担で希望の時は，希望総冊数を原稿に明記すること。

13)　欧文原稿は約 3200 字で 1 印刷ページになり，和文原稿は約 1900 字で 1 印刷ページになります。しかし見出し，図表のまわりなどに余白ができますから，幾分多い目に見積る必要があります。

14)　上記の注意に従わないで書かれた原稿は返却することがあります。

15)　原稿は下記にあて必ず書留便で送つて下さい。

　　　東京都本郷局区内

　　　　　　東京大学理学部植物学教室内

　　　　　　　　　日本植物学会編集幹事

16)　原稿（図共）は雑誌発行後にお返しします。

〇「日本第四紀学会」創立に就いて設立発起人矢部長克氏外から下記の要旨を本会会員に周知方依頼がありましたからお知らせします。

「日本学術会議地質学研究連絡委員会内に第四紀小委員会が設置され，現世を含む最新の地質時代である第四紀の研究を地質学・地理学・古生物学・動物学・植物学・人類学・考古学・地球物理学・地球化学等多くの専門分野に亘る密接な協力のもとに進めてきた従来継続してきた。第四紀小委員会がこの度改組され，新に発足した小委員会は INQUA に対応する国内委員会とし，その活動を研究連絡にのみ限定し，これに対応する研究団体としてここに日本第四紀学会を設立し，旧小委員会の事業の大部分を継承することにきまり，着々その準備が進められている」。

なお入会御希望の方は会費（年 300 円）をそえ，東京都台東区上野公園国立科学博物館内「第四紀学会」（振替口座 東京 13867）へ申込まれたいとのことです。

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第 21 回

日 本 植 物 学 会 大 会

プ ロ グ ラ ム

1 9 5 6

</div>

会　　期　　7 月 9 日（月）―7 月 18 日（水）
会　　場　　北海道大学（札幌市北 8 条西 5 丁目）

<div align="center">

大会名誉会長　　伊 藤 誠 哉
大 会 会 長　　山 田 幸 男

大 会 行 事

</div>

7 月 9 日（月）―7 月 11 日（水）　　見学 E 班
7 月 11 日（水）　　　　　　　　　　評議員会
7 月 12 日（木）　　　　　　　　　　一般講演，記念撮影，部会
7 月 13 日（金）　　　　　　　　　　一般講演，部会
7 月 14 日（土）　　　　　　　　　　特別講演，総会，会長講演，見学 A 班，懇親会
7 月 14 日（土）―7 月 17 日（火）　　見学 C 班
7 月 14 日（土）―7 月 18 日（水）　　見学 D 班
7 月 15 日（日）―7 月 16 日（月）　　見学 B 班
　　　　　（7 月 11 日　藻類学会，13 日　分類学会）
　　　行事の詳細は大会々場に掲示の予定

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大会事務局　　北海道大学理学部植物学教室内

一 般 講 演 時 間 予 定

</div>

日	会場	時　　　　間　　9.30　10　11　12　13　14　15　16　17		
12 日 （木）	A	分　　　類（A1 ～ A9）	（休憩記念撮影）	分　　　類（A10～A24）
	B	細胞，形態，遺伝（B1 ～ B10）		細胞，形態，遺伝（B11～B23）
	C	生理，生化学（C1 ～ C9）		生理，生化学（C10～C23）
	D	生理，生化学（D1 ～ D9）		生理，生化学（D10～D23）
13 日 （金）	A	地理，生態（A25～A34）	（休憩）	地理，生態（A35～A50）
	B	細胞，形態，遺伝（B24～B32）		細胞，形態，遺伝（B33～B46）
	C	生理，生化学（C24～C32）		生理，生化学（C33～C45）
	D	生理，生化学（D24～D32）		生理，生化学（D33～D46）

部　　会　　12日（木）夜　　　　　　生態，形態細胞，菌類談話会
　　　　　　　13日（金）夜　　　　　　生理，分類
特別講演　　14日（土）　9.00 より　┐
総　　会　　14日（土）10.00 より　├ 会場　農学部大講堂
会長講演　　14日（土）11.30 より　┘
懇親会　　　14日（土）17.30 より　会場未定

講 演 プ ロ グ ラ ム

第 1 日　7 月 12 日（木）

【A 会 場】（分 類 学）

9.30— 9.45	(A 1)	好ケラチン性水棲菌類続報	東邦大，理　　大久保　真理子
9.47—10.02	(A 2)	*Protomyces* 属菌の純粋培養について	東京，長尾研　　椿　　　啓 介
10.04—10.19	(A 3)	本邦産 *Cryptococcus* 属菌について	東京，長尾研　　曾 根 田 正 己
10.21—10.36	(A 4)	有柄細菌 *Caulobacter* に関する研究　第1報	東京，長尾研　　増 田 染 一 郎
10.38—10.53	(A 5)	*Allomyces cystogenus* 一変種の生活史に就て	秋田大，学芸　　加 藤 君 雄
10.55—11.10	(A 6)	羽状珪藻 *Synedra Vaucheriae* Kütz. に就て	横浜市大，文理　小 林 艶 子
11.12—11.27	(A 7)	日本産氷雪藻類の研究　（3）	横浜市大，文理　福 島　　博
11.29—11.44	(A 8)	日本産車軸藻類　第9報	東京都立大，理　加 崎 英 男
11.46—12.01	(A 9)	ヨツメモの性と有性生殖	神戸大，理　　　広 瀬 弘 幸

〚綜 合 討 論〛

〚休　　　　　憩〛

13.00—13.10	(A10)	邦産ベニマダラ属の生活環	東京教育大 臨海実験所　千 原 光 雄
13.12—13.22	(A11)	藍藻 *Symploca hydnoides* の分類	京 大，農　　　梅 崎　　勇
13.24—13.39	(A12)	アマノリ類の生活史の研究（続報） オオノノリの繁殖器官について	東北海区水産研　黒 木 宗 尚
13.41—13.51	(A13)	アオノリの海における発芽について	三重大，水産　　瀬 木 紀 男
13.53—14.08	(A14)	日本産ネベリモ属に就て	北大，海藻研　　稲 垣 貫 一
14.10—14.20	(A15)	原始紅藻類ニセウシケノリについて	鹿児島大，水産　田 中　　剛

〚綜 合 討 論〛

14.30—14.45	(A16)	日本産オオミズゴケ類について	広島大，理　　　鈴 木 兵 二
14.47—15.02	(A17)	北海道におけるハリガネゴケ科 (Bryaceae) の蘚類　（予報）	鳥取大，学芸　　越 智 春 美
15.04—15.14	(A18)	ノキシノブ属 (*Lepisorus*) の有性世代	東京教育大，理　川 崎 次 男
15.16—15.26	(A19)	スギナ類についての一考察	北海道黒松内中学　松 川 昌 弘
15.28—15.43	(A20)	日本の石灰岩植物	京 大，理　　＊清 水 建 美 京 大，理　　北 村 四 郎
15.45—15.55	(A21)	花粉の発芽率からみた松柏科植物	大阪市大，理工　栗 本　　喬
15.57—16.12	(A22)	シンジュガヤ類の小穂構造について	東 大，理　　　小 山 鉄 夫
16.14—16.29	(A23)	タヌキモ科植物の腺発生について	東京教育大，理　小 宮 定 志
16.31—16.46	(A24)	樺太植物の総括	北海道学芸大 函 館 分 校　菅 原 繁 蔵

〚綜 合 討 論〛

【B 会 場】（細胞学，形態学，遺伝学）

時間	番号	題目	所属	氏名
9.30— 9.45	(B 1)	子葉の離層分化と遊離·SH基及び生長素	名大，理	堀田康雄
9.47—10.02	(B 2)	オーキシン処理によつて見出されたインゲン子葉の形態形成における役割	東大，理	古谷雅樹
10.04—10.19	(B 3)	インゲン幼胚の組織培養におけるオーキシン処理効果	東大，理／東大，理	古谷雅樹／相馬研吾
10.21—10.36	(B 4)	トウゴマ発芽初期における embryo 組織内のリパーゼ	東大，教養	山田晃弘
10.38—10.53	(B 5)	癒傷組織形成期間中の組織の呼吸について	京大，理	馬場三吾
10.55—11.05	(B 6)	側根原基の組織化学的研究	東大，理	佐藤七郎
11.07—11.17	(B 7)	雌ずいの澱粉と糖について	横浜市大，理	岩波洋造
11.19—11.34	(B 8)	還元分裂中における微量 Zn の組織化学的研究	早大，生物	飯島衞
11.36—11.46	(B 9)	ユキノシタの異なる表皮細胞間の原形質分離限界濃度の比較	熊本大，教育	八戸正夫
11.48—12.03	(B10)	植物細胞における色素透過性の量的研究	東京教育大，理／東京教育大，理	植田利喜造／*村上悟

〔綜 合 討 論〕

〔休　　憩〕

時間	番号	題目	所属	氏名
13.00—13.15	(B11)	裸子植物の受精及び前胚の顕微化学的研究	名大，理	島村環
13.17—13.32	(B12)	羊歯類種子及び前葉体における呈色現象	山形大，教育	伊倉伊三美
13.34—13.49	(B13)	フォイルゲン核アルデヒド反応に関する二，三の知見	京大，理	平岡俊佑
13.51—14.06	(B14)	螢光顕微鏡による植物細胞の研究	東京教育大，理／東京教育大，理	*植田利喜造／前田徴
14.08—14.23	(B15)	ゴアツモリの減数第一，第二分裂中期染色体の退色反応について	慶応大，生物	山崎典子
14.25—14.35	(B16)	カキランの花粉粒分裂の同時性について	慶応大，生物	水野忠欵

〔綜 合 討 論〕

時間	番号	題目	所属	氏名
14.45—15.00	(B17)	帽菌類の diploidisation における移行核について	岡山大，理	木村劼二
15.02—15.17	(B18)	車軸藻の細胞学的研究	立教大，教養	佐々木正人
15.19—15.29	(B19)	X線の高度照射に於ける細胞分裂の特異性(IV)飽和影響後に於ける分裂	函館，湯川小	大山正
15.31—15.46	(B20)	電子顕微鏡による変形菌の微細構造に関する研究 II.	阪大，理	寺田保
15.48—16.03	(B21)	Euglena gracilis の電子顕微鏡的研究	京大，理	植田勝巳
16.05—16.20	(B22)	Oscillatoria princeps の電子顕微鏡的研究	京大，理／京大，理	新家浪雄／植田勝巳
16.22—16.37	(B23)	Bacillus megatherium の紡錘体について	東大，教養	湯浅明

〔綜 合 討 論〕

【C 会 場】（生　理　学）

時間	番号	題目	所属	氏名
9.30— 9.45	(C 1)	密閉状態で起る電載の発根促進効果	千葉県中央電力研	渡部一郎
9.47—10.02	(C 2)	シャジクモ類の細胞間興奮伝導	東北大，理	柴岡孝雄
10.04—10.19	(C 3)	フラスモの膜電位とその時定性	東北大，理	小田健二
10.21—10.31	(C 4)	アサガオ子葉の日長感応	京大，農	今村駿一郎
10.33—10.48	(C 5)	ムシトリナデシコの開花を促進する光週期における2つの段階	京大，農	滝本敦
10.50—11.05	(C 6)	竹笹科の開花の原因	京都，園部高校	高木虎雄
11.07—11.17	(C 7)	花粉管の発芽に及ぼすX線の影響について		池庄司幸江

| 11.19—11.34 | (C 8) | 植物生理学上より見たる日長について | 岡山大
農業生物研 | 高須謙一 |
| 11.36—11.46 | (C 9) | 微量元素と光週性 | 信州大，文理 | 木下哲雄 |

<div align="center">『綜 合 討 論』</div>

<div align="center">［休　　　憩］</div>

13.00—13.10	(C 10)	*Porphyra* 果胞子の石灰性への穿孔性に就て	大阪市大，理工	尾形英二郎
13.12—13.27	(C 11)	気孔の夜間開孔と葉の有機酸との関係について	金沢大，理 新潟大，教育 名城大	西田晃二介 相馬悌 日比野信一
13.29—13.44	(C 12)	フラスモ節間細胞の構造と原形質流動に就て	新潟大，教育	相馬悌一介
13.46—14.01	(C 13)	ヘチマの溢泌液中における P³² の形態の変化	金沢大，理 金沢大，理	玉井直郎 西田晃二人
14.03—14.18	(C 14)	葉の P³² 蓄積に及ぼす光の影響	金沢大，理 金沢大，理	西田晃二雄 玉井直剛
14.20—14.35	(C 15)	オシダの胞子の発育におよぼす光の影響	東京教育大 東京都立大，理	石川房男 大房剛
14.37—14.52	(C 16)	糸状菌胞子の諸種方法による殺滅について	お茶の水女大	大槻虎男
14.54—15.04	(C 17)	*Chlamydomonas* sp. の生長	神戸大，理	高橋和民
15.06—15.16	(C 18)	麺麭赤黴菌子実体形成に及ぼす窒素源の影響について	帯広畜大	伊藤太郎

<div align="center">『綜 合 討 論』</div>

15.30—15.45	(C 19)	メタセコイアの吸水と培養液滲透価との関係	大阪市大，理工	河原晨
15.47—15.57	(C 20)	水度傾斜培地に於ける抗酸菌の増殖について	広島大，理	西尾隆昌
15.59—16.09	(C 21)	生長週期に於ける結合水量と滲透価の関係	広島大，理	高沖武
16.11—16.21	(C 22)	水度傾斜培地における葉温の周期的変化について	広島大，理	本田稔
16.23—16.33	(C 23)	能動的吸水の静的解釈と動的解釈	広島大，理	福田八十楠

<div align="center">『綜 合 討 論』</div>

<div align="center">［D 会 場］（生 理 学）</div>

9.30— 9.40	(D 1)	イネ鞘葉の脱水素酵素	東北大，理	大脇頼子
9.42— 9.57	(D 2)	*Euglena* のチトクロームについて	東大，理	西村光雄
9.59—10.09	(D 3)	*Phytophthora infectans* (Mont.) de Bary の発育並びに寄生体侵入に伴うチトクロームオキシダーゼの消長について	島根農大 植物病理	山本昌木
10.11—10.26	(D 4)	コウジカビの燐酸代謝について (II) mitochondria の酸化系	阪大，理 阪大，理 阪大，理	今本文男 義佐貫一三男 *奥貫一耕男
10.28—10.43	(D 5)	コウジカビの燐酸代謝について (III) Adenylate kinase の役割について	阪大，理 阪大，理 阪大，理	*義佐本文三男 今貫佐一耕男 奥内一夫
10.45—11.00	(D 6)	変形体の酸溶性高エネルギー燐酸化合物について II.	阪大，理 阪大，理	竹内郁司 秦野節夫
11.02—11.17	(D 7)	クロレラの醋酸代謝に関する研究	東大，理	小笠原和子
11.19—11.29	(D 8)	クロレラの尿素及びアンモニアの代謝と燐酸の関係について	東工大，生物	服部明彦
11.31—11.46	(D 9)	耐熱性藍藻 *Cyanidium* の代謝生理（第二報）	東大，理	福田育二郎

<div align="center">『綜 合 討 論』</div>

<div align="center">［休　　　憩］</div>

13.00—13.10	(D 10)	糸状菌による蝋，パラフィンの分解	愛媛大，文理	宮本義男
13.12—13.22	(D 11)	*Aspergillus niger* のパラフィン分解に於ける有機酸の生成	愛媛大，文理 愛媛大，文理 愛媛大，文理	古川満男 *三戸昭男 宮本義男
13.24—13.39	(D 12)	糸状菌の蝋上に於ける発育について（続報）	お茶の水女大	今井百合江子

✓ 13.41—13.51	(D 13)	大豆及びカゼイン培地に於ける *Streptomyces griseus* の呼吸について	{科 研 久井 保 秀行 雄 科 研 上 山 弘行 雄
✓ 13.53—14.08	(D 14)	*Streptomyces griseus* の末端酸化系について	{科 研 中井 山上 弘行 美雄
✓ 14.10—14.25	(D 15)	結核菌におよぼす高級脂肪酸の影響について——その殺菌作用と形態変化とに関連して	福島医大, 細菌 南 一 守
14.27—14.37	(D 16)	酵母の同調的増殖中にみられる生理的活性の変化について	東工大, 生化 野 宗 嘉 明

〖綜 合 討 論〗

✓ 14.50—15.05	(D 17)	水稲フォスフォリラーゼ及びアミラーゼの性質と種実の発育過程に於ける変化	{農 技 研 相 見上 靈 三高 農 技 研 村 靈 三子
15.07—15.22	(D 18)	水稲種実の発育過程に於けるフォスフォリラーゼ, 燐酸, 等電点, 澱粉等の分布と消失に関する組織化学的研究	{農 技 研 相 見上 靈 三子 農 技 研 藤 巻 和
15.24—15.39	(D 19)	水稲種実における澱粉の集積機構に関する細胞生理学的研究	{農 技 研 相 見上 靈 三高 農 技 研 村 上中 昭 浩一
15.41—15.56	(D 20)	植物の伸長と酵素活性の消長	{農 技 研 *村 農 技 研 松林 武 夫
15.58—16.13	(D 21)	種子発芽時の生理化学的研究 (2)	{阪 大, 理 田 川 邦 一 男 阪 大, 理 *奥 貫 一
16.15—16.30	(D 22)	種子の発芽時に於ける呼吸に及ぼすクマリンの影響	熊本大, 理 石 川 一重 夫
16.32—16.42	(D 23)	光週処理に伴うある代謝系の活性度変化	信州大, 文理 柴 田 治

〖綜 合 討 論〗

第 2 日　7 月 13 日（金）

【A 会 場】（地理学, 生態学）

9.30— 9.43	(A 25)	Interspecific correlation による着生植物の群落構造解析（続）	九 大, 理 小 村 精
9.45— 9.58	(A 26)	着生植物の樹上分布と日補償点について	九 大, 理 小 谷 信 矢
10.00—10.10	(A 27)	*Ulota crispula* の生態	九 大, 理 細 川 隆 英
10.12—10.25	(A 28)	岩上着生植物群落の生態学的研究	神戸大, 教育 中 西 哲
10.27—10.40	(A 29)	北海道の蘚類 (2)　ミズナラに着生する蘚苔類の一考察	{北 海 道 *齋 藤 実一 富良野高校 佐々木 太 蔵
10.42—10.52	(A 30)	遼東半島沿岸の海藻相について	新潟大, 理 野 田 光 蔵
10.54—11.04	(A 31)	中国地方に於けるブナ林と常緑広葉樹林との推移帯植生について	広島大, 理 佐々木 好 之
11.06—11.19	(A 32)	宮島のモミ, ツガ林について	広島大, 理 奥 富 清
11.21—11.34	(A 33)	ドクダミの群体形成についての考察	東北大, 理 飯 泉 茂
11.36—11.49	(A 34)	北海道の雑草群落	横浜国立大, 学芸 宮 脇 昭

〖綜 合 討 論〗

〖休 憩〗

13.00—13.13	(A 35)	裏日本植物の分布型	東 大, 理 小 野 幹 雄
13.15—13.28	(A 36)	日本産針葉樹の温度分布	大阪市大, 理工 吉 良 竜 夫
13.30—13.43	(A 37)	麦畠の微気象について	京大, 理 村 田 茂 三
13.45—13.58	(A 38)	岡山県八束村の珪藻土について	京都工繊大 奥 野 春 雄
14.00—14.13	(A 39)	遺体からみたチョウセンゴヨウ	大阪市大, 理工 三 木 茂
14.15—14.28	(A 40)	新しい方法による生長曲線の解析	大阪市大, 理工 生 嶋 功 人
14.30—14.43	(A 41)	個体密度の自然間引	{大阪市大, 理工 *小 川 房 二 大阪市大, 理工 依 田 恭

〖綜 合 討 論〗

14.55—15.08	(A 42)	マツ科に於ける種子蛋白の変異	京 大，理 京 大，理	岡 田 幸 郎 桃 谷 好 英 馬 相 寛 吉 雄
15.10—15.23	(A 43)	仙台附近鮮新世亜炭の花粉分析（第２報）	東北大，理	
15.25—15.38	(A 44)	山形県系炭田の花粉分析学的研究──特に県内に点在する二，三炭鉱の花粉分析	山 形 県 新庄北高校	高 橋 信
15.40—15.53	(A 45)	植物の健康並びに性能の診断法としての根系呼吸測定法	東 大，理 小田原女高	高 橋 基 生 松 浦 正 郎
15.55—16.08	(A 46)	植物の生長経過別による培養液組成の根系呼吸に及ばす影響	東 大，理 野 口 研 野 口 研	高 橋 基 男 高 谷 義 生 青 本 和 基
16.10—16.20	(A 47)	切断根系の養分吸収並びに呼吸の不自然性	東 大，理	高 橋 基 勇 高 橋 基 孝
16.22—16.32	(A 48)	根系呼吸に対する土壌成分の温度補償性と植物の分布	東 大，理 横浜女商高 浦 賀 中	高 橋 基 生 石 黒 コ 生 桝 井 喜 昭
16.34—16.44	(A 49)	各種水耕液の性能判定と根系呼吸	東 大，理 横 浜 中	高 橋 基 平 田 ノ
16.46—16.59	(A 50)	青枯水田土壌の特色	東 大，理 兵 庫 農 試	高 木 高 青

〔綜 合 討 論〕

〔Ｂ 会 場〕 （細胞学，形態学，遺伝学）

9.30— 9.45	(B 24)	題 未 定	愛媛大，教育	神 野 太 郎
9.47—10.02	(B 25)	*Chrysanthemum Yoshinaganthum* における生態型の分化に関する形態学的細胞学的研究	広島大，理	田 中 隆 荘
10.04—10.19	(B 26)	*Chrysanthemum japonense* の種内倍数と地理的分析	広島大，理	下 斗 米 直 昌
10.21—10.31	(B 27)	*Aster* 属植物の核型分析	神戸大 御影分校	藤 原 悠 紀 雄
10 33—10.48	(B 28)	コムギ近縁種としてのカモジグサの細胞遺伝学的研究	遺 伝 研 京 大，農 遺 伝 研	松 村 清 二 村 松 幹 夫 阪 本 寧 男
10.50—11.05	(B 29)	核置換小麦花粉粒の発育限度（仮）	京 大，農	木 谷 義 明
11.07—11.22	(B 30)	タバコ属の細胞遺伝学的研究	遺 伝 研	竹 中 要
11.24—11.34	(B 31)	蘚類数種の染色体数	大分大，学芸	三 宮 正 信
11.36—11.46	(B 32)	Reed Canary Grass に於ける異数性と核型	東京農大 茂 原 分 校	柴 田 寛 三

〔綜 合 討 論〕

〔休 憩〕

13.00—13.10	(B 33)	題 未 定	九 大，理 九 大，理	*稲 田 朝 次 岩 佐 正 一
13.12—13.27	(B 34)	果樹の接木による交配	北海道学芸大函館分校 北海道学芸大函館分校 北海道学芸大函館分校	田村半吾 菅原繁蔵 *浅利政俊
13.29—13.44	(B 35)	ヤクシソウ×アキノノゲシの F₁ における染色体減少の原因	東京都立大，理	酒 井 文 三
13.46—13.56	(B 36)	ミツイシコンブの游走子嚢発生と游走子形成	岡山大，理	猪 野 俊 平
13.53—14.13	(B 37)	ヒラタケの発生	東大，教養	寺 川 博 典
14.15—14.30	(B 38)	蘚類のさく歯の発生学的研究 V. イクビゴケのさく歯の発生について	島根大，文理	斎 藤 真 太 郎
14.32—14.42	(B 39)	無胞子生殖的に再生されたシダについて (2)	名 大，理	高 橋 千 裕
14.44—14.59	(B 40)	前葉体細胞の再生能及び分裂能について	名 大，理	伊 藤 道 夫

〔綜 合 討 論〕

15.10—15.25	(B 41)	インゲンの胚発生（第２報）	東 大，理	竹 内 正 幸
15.27—15.42	(B 42)	アマの子房構造	東大，教養	木 村 陽 二 郎
15.44—15.54	(B 43)	ラン科の地下器官の発生	名大，教養	熊 沢 正 夫
15.56—16.11	(B 44)	ツツジ科の生長点	東 大，理	原 襄

16.13—16.28	(B 45)	*Xenoxylon latiporosum* に関する二，三の知見	東　大，理 東　大，理	瓦　倉　理　俊 小　田　文　謙	次吾謙
16.30—16.45	(B 46)	紡錘体に関する二，三の問題	東　大，理	和　田　文	吾

〔休　　憩〕

【C 会 場】（生理学，生化学）

9.30— 9.45	(C 24)	リンゴのポリフェノールオキシダーゼ	富山大，理	高　桑	昇
9.47—10.02	(C 25)	タバコの葉のポリフェノールオキシダーゼ	東　大，理 東　大，理	服　部　静　夫 *代　谷　次　夫	
10.04—10.19	(C 26)	葉緑体蛋白の研究　III	九　大，理	千　葉　保　胤	
10.21—10.36	(C 27)	$C^{14}O_2$ を用いた炭酸固定の研究　（第六報）前照射によつて生成した光化学的還元物質の定量	東　大，理 徳川生物研 〃 〃 〃	宮　地　重　遠 加　藤　哲　也 広　川　宮　康 田　　　博 	旭
10.38—10.58	(C 28)	光化学的燐酸転移	東　大，理	三　井	旭
10.55—11.10	(C 29)	濁度滴定（新しい蛋白定量分析法）に関する二，三の問題	京　大，理	桃　谷　好　英	
11.12—11.22	(C 30)	百合科植物の生理 II. チューリップの一品種の花弁におけるアントチアニンの研究	富山大，理	堺　　　惠　美	
11.24—11.39	(C 31)	チューリップの一品種「エクリプス」のアントチアン	富山大，理	柴　田　萬　年	
11.41—11.56	(C 32)	*Oscillatoria princeps* の DNA について	京　大，理	石　田　政　弘	

〔綜　合　討　論〕

〔休　　憩〕

13.00—13.10	(C 33)	空中窒素固定能を有する藍藻類の稲の收穫に及ぼす影響　(III)	成　城　大	渡　辺　　　篤	
13.12—13.27	(C 34)	藍藻類の生理学的研究　（其の一）	南山大学 南山大学	細　井　暁　光 野　村　昭　之	助
13.29—13.39	(C 35)	Vernalization と生長点における核酸の消長	九　大，農	西　　　克　久	
13.41—13.56	(C 36)	トウゴマ貯蔵油脂の発芽期における利用状態	東大，教養	山　田　晃　弘	
13.58—14.13	(C 37)	地衣藻培養の際にあらわれた細菌の培養について	奈良女大，理	西　村　公　臣	

〔綜　合　討　論〕

14.25—14.40	(C 38)	麴菌の分生胞子の色素に関する研究（第二報）	東　大，農 東　大，農	高　木　嘉　昌 坂　口　譲　一	郎
14.42—14.52	(C 39)	ハッショウマメに於けるジオキシフェニールアラニンについて	東　大，理 東　大，理	服　部　静　夫 *駒　鎭　穆	
14.54—15.09	(C 40)	緑藻細胞膜質の多様性について	東京教育大，理 東京教育大，理	三　輪　知　雄 入　来　義　彦	造
15.11—15.26	(C 41)	ガラガラ科石灰藻のカルシウムと酸脱水素酵素について	東京学芸大 東京教育大，理	古　谷　庫　雄 三　輪　知　至	大
15.28—15.43	(C 42)	題　未　定	宮崎大，学芸	中　山　至　大	
15.45—16.00	(C 43)	オジギソウ葉枕の細胞生理学的研究（第七報）	東京女子大	鳥　山　英　雄	
16.02—16.12	(C 44)	アメリカヤマゴボウの葉緑体の異状について	東京女子大	鳥　山　英　雄	

〔綜　合　討　論〕

【D 会 場】（生　理　学）

9.30— 9.45	(D 24)	銅抵抗性コウボ変異細胞の起源　(I)	京　大，理 京　大，理	持　塚　洵　治 芦　田　忠　譲	
9.47— 9.57	(D 25)	銅抵抗性コウボ変異細胞の起源　(II)	京　大，理 京　大，理	芦　菊　池　田　譲　寿 芦　田　　　治	

時間	番号	題目	所属	演者
9.59—10.09	(D 26)	銅抵抗性コウボの硫黄代謝 (I)	京大，理 京大，理	蘆田信譲 夫 内田貴六 治
10.11—10.21	(D 27)	銅抵抗性コウボの硫黄代謝 (II)	京大，理 京大，理	蘆原井浄 雄 今榊田譲 子
10.23—10.38	(D 28)	酵母におけるW変異菌とその銅適応における意義	大阪市大，理工	柳島直純 治
10.40—10.55	(D 29)	酵母変異菌のイオン交換性 (I)	大阪市大，理工	平岡英徹 彦
10.57—11.12	(D 30)	酵母変異菌のイオン交換性 (II)	大阪市大，理工	高田山護 一
11.14—11.29	(D 31)	コウボのアミノ酸プールに対する諸毒物の影響	京大，理 京大，理	村山田 夫 蘆 勝 郎治
11.31—11.41	(D 32)	酵母の銅耐性と窒素源	甲南大，文理	荒 豐

〔綜 合 討 論〕

〔休　　憩〕

時間	番号	題目	所属	演者
13.00—13.10	(D 33)	題 未 定	大阪市大，理工	近衞康 也
13.12—13.27	(D 34)	ギベレリンとオーキシンとの相互作用及びギベレリンの伸長，呼吸及び吸水に対する影響	京大，理	加藤次 郎
13.29—13.39	(D 35)	長日植物ベッチ (Vicia sativa) の光週反応に及ぼす NAA の影響		賀来章 輔
13.41—13.56	(D 36)	Auxin処理によるムシトリナデシコの抽苔について	京大，農	小西通 夫
13.58—14.08	(D 37)	オーキシン作用とペクチン質酵素	京大，理 京大，理	小林静譲 子 蘆田田 治
14.10—14.20	(D 38)	マレイン酸ヒドラジドの作用機作に関する一考察	神戸大，理 神戸大，理	須田省勝 三 新田 光 之
14.22—14.37	(D 39)	トランス桂皮酸はアンチオーキシンか (2,4,o-トリクロロフェノキシ醋酸との比較)	東北大，理 東北大，理 東北大，理 東北大，理	*長尾野昌晴 子 草野辻脇 望 尾大田赤芳 子 増 雄
14.39—14.49	(D 40)	オーキシン作用の原形質学的研究	愛媛大，文理	増田 雄
14.51—15.01	(D 41)	イヌマキの胎生種子及び休眠種子内における生長作用物質の動静	奈良女大，理 奈良女大，理	小清水卓 二 *吉原朝 子
15.03—15.18	(D 42)	茎の極性と生長素	東京学芸大	小林菫寿 男
15.20—15.30	(D 43)	二，三緑藻のつくる生長素物質について	神戸大，理 神戸大，理	岩田修造 三 須田省 止
15.32—15.42	(D 44)	題 未 定		中沢敬 野
15.44—15.54	(D 45)	フジアザミの炭水化物について	埼玉大，文理	村上 進
15.56—16.06	(D 46)	シラネアオイの根茎の成分	星薬大	木下広

第 3 日　7 月 14 日（土）

特 別 講 演

時間	題目	所属	演者
9.00—10.00	藻類の起源と系統	東大，理	前川文夫

会 長 講 演

時間	題目	所属	演者
11.30—12.30	シソ科及びゴマノハグサ科の少糖類について	東大，理	服部静夫

Contributio ad Floram Asiaticam Novam-Guineam inclusem

acutore Tetsuo Koyama*

小山鉄夫：アジアの植物への寄与

Carex hypolytrifolia T. Koyama, spec. nova ad sectionem *Mapaniifoliae;* omnibus speciebus in hac sectione huc usque descriptas habitu multo minore, foliis brevioribus angustioribusque flaccidis tenuibus, paniculis partialibus laxioribus ita rachi visibili valde dissimilis est. (Fig. 1)

Perennis haud caespitans, *rhizomate* crassiusculo lignoso collo ascendente squamis fuscis obtecto, radicibus validis. *Folia* omnia radicaria ex unico rhizomate circa 7 fascicularia late linearia usque linearioblanceolata 28–52 cm longa medio 15–20 mm lata laete viridia flaccida plana praeter margines minute scabres laevia apice

Fig. 1. *Carex hypolytrifolia*, n. sp. a. spicula; b. bracteola spiculae; c. prophyllum basis spiculae; d. squama floris foeminei; e. utriculus; f. nux cum stylo; g. os rostri utriculi.

subsensim breviuscule acuta basi in vaginas breves antice fulvohyalinas dorso purpurascentinervatas gradatim attenuantia. *Vaginae* basilares aphyllae vaginiformes membranaceae fuscae non fibrososolutae. *Culmus* centralis e fasciculo foliorum solitarius erectus 4–5 dm altus graciles 1–1.5 mm crassus obtuse trigonus laevissimus. *Inflorescentia* erecta paniculata decomposita subcontigua oblonga 15 cm longa 4 cm lata; *paniculae partiales* 3 singulae ovoideae erectae 3–6 cm longae 2.5–4 cm latae densiusculae 3–5-spicatae longe exsertopedunculatae, *pedunculis* acute triquetris ad

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* Botanical Institute, Faculty of Science, University of Tokyo. 東京大学理学部植物学教室

angulo hirtoscabris, *rachi* acute triquetra scabrangula ; *bracteae* vaginantes inferior sub-
foliacea, laminâ ad 8 cm longâ 6 cm latâ paniculam secundariam suam superante,
vaginâ ad 2.5 cm longâ, superior subspathacea paniculâ suâ multo previor. *Spicae*
ellipsoideae pluri-(3-7-)spiculosae patentes subsessiles, *rachi* e prophyllo ochreiforme
tenuimembranaceo enatâ hispidangulâ, *bracteolis* squamiformibus dorso breviter
hispidis apice scabroaristatis. *Spiculae* androgynae rectangulariter divergentes
ovoideae 8-10 mm longae maturitate 5-7 mm crassae dense multiflorae, parte foe-
mineâ 3-6-florâ eâ masculâ ellipsoideâ multiflorâ breviore ; *prophylla* spathaceoutri-
culiformia membranacea fulva superne sparsim hispidula, *bracteolis* eae spicarum
similibus. *Squamae* foemineae lanceolatae vel oblongolanceolatae 3.8-4.5 mm longae
0.8-1.2 mm latae membranaceae inferne glabrae superne minute hirsutae dilute
fulvae a basi ad apicem acutiusculam hyalinam utrinque sensim attenuantes dorso
subaequliter pluri-(ad 17-) nerviae. *Utriculi* maturitate patentes squamam superantes
6-7 mm longi 1.5 mm lati membranacei olivaceovirentes praeter costas 2 tenuiter
multinervosi a medio ad apicem hispidi, e parte inferiore rhomboideo-ovale vere tri-
gonâ basi subito cuneatocontractâ subestipitatâ apice etiam subito attenuatâ in
rostrum gracile perlongum 3-3.5 mm longum 0.2 mm latum primo erectum demum
plerumque flxuosum ad angulos sparasim spinulosohispidum transeuntes, *ore* hyalino
scabrido profunde oblique fisso. *Nux* arcte inclusa late rhomboidea 2-2.2 mm longa
1.5 mm lata vere triquetra facie concaviuscula atrofusca ad angulos prominentes
flavida apice basique abrupte cuneatocontracta ; *stylus* longus gracilis flexuosus basi
breviter conicoincrassatus, stigmatibus 3 recurvis brevibus papulosis.

A N N A M : Bun Mo, 800m. alt Leg. B. Hayata, sin. num.! —holotypus in TI.
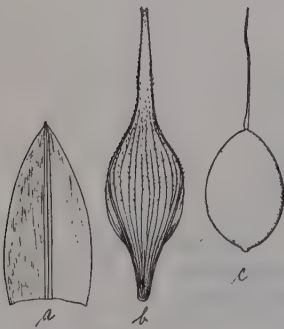
Fig. 2. *Carex megacarpa*,
n. sp. a, squama floris
foeminei ; b. utriculus ; c.
nux. (Kostermans, 6231)

Carex megacarpa T. Koyama, spec. nova in
sectione *Graciles;* proxime ad hanc speciem accedit
C. nachiana Ohwi ex Japonia descripta, tamen a
planta javanensi utriculis late ovalibus 3.5-4 mm
longis, habitu elatiore etc. satis distinguitur, et *C.
autumnalis* Ohwi, etiam planta Japoniae australis,
est valde distincta ab hac spiculis sexu distinctis,
utriculis vix 3 mm longis.——? *C. brunnea* Thunb.
var. *dolichocarpa* Nelmes in Kew Bull. **1950**: 201
(1950), e descriptione. (Fig. 2)

Perennis, *rhizomate* abbreviato lignoso perdense
caespitoso, radicibus validis. *Culmi* erecti 60-100
cm alti vere triquetri sursum scabridi nutantes inferne laeviusculi remote
1-3-foliati basi vaginis obtecti. *Vaginae* basilares aphyllae vel laminatae fuscobrun-
neae dorso demum in fibras sparse solutae antice membranaceae dein in fibras
reticulatim fissae. *Folia* radicalia culmorumque linearia 1/2-2/3-culmo aequilonga
2-3 mm lata plana vel subconduplicata scabrida apice longissime acuminata basi

in vaginas longas (5–7 cm in foliis culmorum) antice tenuimembranaceas fulvas reticulatim nervulosas vix attenuata. *Spiculae* 8–13 superiores singulae geminaeve inferiores in paniculis secundariis perlaxis dispositae paniculam gracilem formantes, omnes androgynae oblongo- vel vere cylindricae 1.5–3 cm longae maturitate 3–4 mm crassae densiuscule pluriflorae, parte foeminea ea mascula 2-4-plo superante, *pedunculo* gracili scabro longe exserto. *Bracteae* inferiores subfoliaceae panicula partiali sua paullo longiores basi longe vaginantes, caeterae bracteolaeque spathaceae margine anguste fulvomembranaceae apice interdum aristatae. *Squamae* foemineae ovatoellipticae vel ellipticae 3–3.5 mm longae ca. 1.5 mm latae tenuiter membranaceae medio minute fuscolineolatae latere fuscotinctae margine peranguste hyalinae apice acutiusculae obtusiusculaeve, *carina* late virente uninervia. *Utriculi* squama longiore latioreque suberecti ovato-oblongi vel oblongeoelliptici 4–5.5 (–6) mm longi medio 1.3–1.5 mm lati planoconvexi vel biconvexi membranacei distincte plurinervosi maturitate fulvi, praecipue supra medium marginibusque versus apicem hispiduli, deorsum subsensim contracti in stipitem glabrum 1/2–2/3 mm longam, sursum sensim contracti in *rostrum* rectum 1.5–2 mm longum antrorsim hispiduloscabrum, *ore* hyalino oblique fisso. *Nux* arcte inclusa ovalis 2.5–3 mm longa 1.4–1.7 mm lata biconvexa flavens apice basique rotunda mucronulata, *stylo* longo recto basi subaequali, stigmatibus 2 rostro paullo longioribus.

J a v a: in sylva montis, Lawn super Tjimcrosewu, Sarangan. Leg. J. H. Kern, 8672 !—holotypus in TNS. Sine loco speciali. Leg. Kostermans, 6231 !.

Carex (*Ischnostachyae*) **ischnostachya** Steudel

var. **fastigiata** T. Koyama, var. nova, a typo diversa utriculis non vel brevissime rostratis 2.8–3.5 mm longis, spiculis superioribus 3–5 apice culmi fastigiatis. *C. subtumida* Ohwi ab hac recedit habitu robustiore, culmo valde scabro, rhizomate longe stolonifero etc.

R y u k y u: m. Tanimadake, Hajimura in ins. Okinawa. Leg. T. Amano, 7586 !—hyiotypus in TI.

Carex (*Stellulatae*) **omiana** Franchet et Savatier

var. **perileia** (S. T. Blake) T. Koyama, stat. nov.—*C. perileia* S. T. Blake in Journ. Arn. Arbor. **28**: 102 (1947); Nelmes in Reinwardtia **1**: 441 (1951). Distrib. in Nova-Guinea endemica.

Eriocaulon Omuranum T. Koyama, spec. nova e sectione *Spathopeplus*; *E. Takae* Koidz. et *E. hondoensi* Satake proximum videtur, tamen ab hoc bracteis involucri apice acutissimis non dentatis floribus duplo superantibus medio latiuscule viridibus et ab illo habitu multo minore, petalis intus glaberrimis recedit. (Fig. 3)

Annuum gracile acaulescens, *radicibus* fibrosis albis transverse septatis. *Folia* pauca linearia 1/2–1 mm lata 5.5–12 cm longa flaccida circiter 4-nervata sep-

tatonodulosa versus apicem longe acuminatam gradatim attenuantia. *Pedunculi* 1–4 ex unica planta erecti capillares 10–15 cm alti vix torti 4-costati, *costis* anguste membranaceis. *Vaginae* basilares basem pedunculi perlaxe circumdantes cylindricae 1.5–4.5 cm alti 1–1.7 mm lati septatonodulosae apice hyalinae oblique fissae. *Capitulum* turbinatum 4.5–5.5 mm altum 4–6 mm in diametro omnino glaberrimum 9–17-florum; *bracteae* involucrantes (4–) 5–6 erectopatentes lanceolatae vel anguste lanceolatae 2.5–6 mm longae 0.8–1.2 mm latae sursum gradatim attenuatae apice longe subulatocuspidatae medio late virides membranaceae marginibus utrinque latiuscule albohyalinae; *receptacrum* glabrum. *Flores foeminei* marginales cum stipite circiter 1/2 mm longo 3.7 mm longi; *bractea* lanceolata oblongolanceolatave hyalina pallida 2.5 mm longa apice acutiuscula; *calyx* ovalis 2.3 mm longus hyalinus pallidus obsoletissime trinervulosus ex toto glaberrimus apice contractus tridentatus, dentibus deltoideis centrale quam laterales 2 breviore angustioreque omnibus glabris; *petala* 3 libera lanceolata vel ovatolanceolata alba 3.5 mm longa calycem paullo excedentia basi subabrupte contracta stipitata intus extraque glabra apice acutiuscula mucronata glandula unica coronata; *ovarium* triloculare, *stylo* longo recto, stigmatibus 3. *Flores masculi* centrales 1.7 mm longi; *bractea* oblanceolata hyalina pallida flore paullo brevior apice rotunda; *calyx* spathiformis hyalinus pallidus apice obtusus vel leviter emarginatus omnino glaber; *corolla* subteres apice trilobata, *lobis* aequalibus oblongis obtusis; *stamina* 6 lobis corollae longiora, *antherae* globosae nigrae.

J A P O N I A: circa lacum Shirakabako, Honshu. Leg. Toshiro Omura, 6 aug., 1951 !— holotypus in TNS.
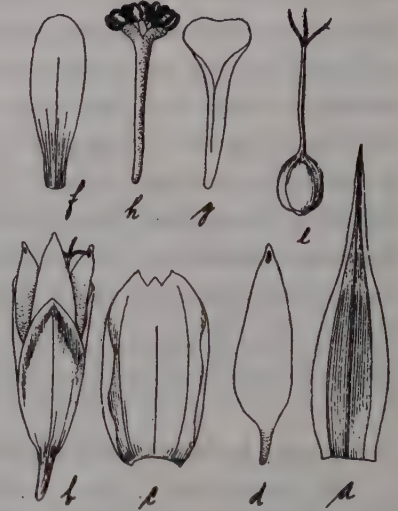
Fig. 3. *Eriocaulon Omuranum*, n. sp. a. bractea involucri; b. flos foemineus cum bractea sua; c. ejusdem calyx; d. ejusdem petalum; e. pistilum; f. bractea floris masculi; g. calyx floris masculi; h. corolla floris masculi cum staminibus. Omnes e typo delineabantur.

Scirpus Ohwianus T. Koyama, spec. nova e vicinia *Scirpi juncoidei* Roxb. a qua nuce obovata non orbiculare, spiculis pluribus densius floriferis culmis robustioribus ample distinctus—' *S. juncoides* (non Roxb.)': Ohwi in Mem. Coll. Sci. Kyoto Imper. Univers. ser. B, **18** (1) : 113 (1943), pro parte, excl. var. *Hotarui* Ohwi. (Fig. 4)

Herba annua caespitans fere erhizomata, *radicibus* fibrosis multis fuscescentibus. *Culmi* erecti 3–6 dm alti 1.2–4 (raro ad 5) mm crassi tereti obscure pluricostulati laeves laete virides opaci basi vaginis paucis obsiti. *Folia* omnia in vagias aphyllas reducta. *Vaginae* basilares inferiores tenuiter membranaceae fuscae squamiformes usque subspathaceae apice profunde oblique fissae obtusae vel plus minus emar-

ginatetae, superiores cylindricae, summa ad 13 cm longa dorso viridis antice hyalina ore oblique secta mucronata. *Inflorescentia* pseudolateralis capitatocontracta (1–) 2–9 (–12)-spiculosa. *Bractea* unica teres culmum continuans erecta 5–15 cm longa inferne antice unisulcata. *Spiculae* oblongocylindricae radiantes 9–18 mm longae 3.5–5 mm in diametro dense pluriflorae fulvovirentes. *Squamae* imbricatim dispositae spissae ovales 3.5–4 mm longae 1.8–2.7 mm latae valde naviculares membranaceae latere utrinque fulvotinctae apice rotundatae otusae vel emarginatae mucronatae margine latiuscule albohyalinae inferne aequaliter tenuinerviae, *costa* late viridi 1–3-nervia. *Nux* obovata subbiconvexa 1.8–2 mm longa circiter 1.5 mm lata basi abrupte attenuata obdeltoidea apice rotundocontracta mucronata facie
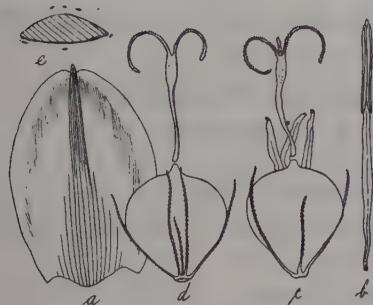
Fig. 4. *Scirpus Ohwianus*, n. sp.
a. squama; b. stamen; c. nux cum setis, filamentis et stylo, postice visa; d. eadem cum setis styloque a dorso visa; e. sectio transversalis nucis (e typo).

nitida plus minus zonatorugulosa maturitate nigricans. *Setae* hypogynae 4–6 tenues fluvescentes praeter partem basilarem retrorsim subdense spinulososcabrae, 4 nucem aequantes paullo superantesve, ceterae cum 1/2–2/3-nuce aequilongae. *Stylus* rectus 2–2.2 mm longus basi aequalis sub stigmatibus subcompressus, *stigmatibus* 2 sed saepe cum rudimento brevi unico ita ut videtur trifidis. *Stamina* 3, *filamentis* planis, *antheris* 1.3 mm longis, *connectivo* deltoideo breve.

J A P O N I A: circa Yatsumi, Prov. Kadzusa. Leg. T. Koyama, 14591!—holotypus in TNS. Distrib. sp. Japonia (Honshu, Kyushu), Ryukyus.

Nota: Haec species cum *Scirpo Hotarui* Ohwi saepissime confusa est, tamen ab illo funditus diversa est:

A. Culmus crassior obsolete pluricostulatus; spiculae 3–7; oblongae breviter cylindricaeve; nux biconvexa, styli ramis 2. 　　　 S. *Ohwianus*, n. sp.

AA. Culmus gracilior laevissimus non costulatus nec angulatus in vivo plus minus nitidulus; spiculae 1–3 ovoideae globoso-ovoideaeve; nux adpresse triquetra, facie postica concaviuscula, styli ramis semper 3. 　　　 S. *Hotarui* Ohwi

Poa finitima T. Koyama, spec. nova ad seriem *Pratenses*, a *Poa shinanoana* Ohwi lemmate antherisque brevioribus et habitu minore praecipue distat. (Fig. 5)

Gramen perenne, *stolonibus* gracilibus perlongis horizontaliter repentibus fibris fuscis sparse obtectis. *Culmus* ex unico rhizomate solitarius erectus 45–55 cm altus 2–3-nodosus basi geniculatus circ. 1 mm crassus vaginis fuscis fulvisve otsitus, remote pauci-(saepissime 2)-foliatus laevis plus minus nitidulus. *Folia* linearia, *laminis* glabris vagina brevioribus 2–2.5 mm latis usque ad 6 cm longis, *vaginis* infra medium antice connatis 5–12 cm longis internodio brevioribus, *ligulis* hyalinis rotundis fere 1 mm longis. *Panicula* oblongo-ovata vel oblonga densiuscula sub-

erecta 6–12 cm longa 3.5 cm lata, *ramis* (2–) 3–5-nis capillaribus flexuosis scabrius-
culis post anthesi patulis 3–5-spiculatis basi nudis. *Spiculae* compressae 3–4-florae cine.
reovirentes vel purpurascenticinereae 5.5–7 mm longae; *glumae*
steriles lanceolate acuminate 3–3.5 : 3.7–4 mm longae trinervatae,
nervis 2/3–3/4-glumae attingentibus, *carina* sursum minute
scabra; *lemmata* anguste oblonga vel lanceolato-oblonga 4–4.5
mm longa apice latiuscule hyalina acutiuscula praeter carinam
minute scabram latere utrinque 2-nervosa basi cuneatoangustata
lana longa cincta, carina ad 1/2 nervis marginalibus ad 1/3
proprius usque pilosis, nervo intermedio nudo; *paleae* lemmate
paullo breviores 3.5–4 mm longae hyalinae apice rotundotrun-
catae; *antherae* lineares 1.5–1.7 mm longae.

Fig. 5. *Poa fini-
tima* n.sp. Spicula
(e typo).

J A P O N I A: in resione alpina montis Kiso-Komagatake,
prov. Shinano. Leg. K. Machida, s. n. !—holotypus in TI.

Uncinia Ohwiana T. Koyama, spec. nova distinctissima ex
affinitate *U. caespitosae* quae spiculis multo longioribus perlaxe florentibus valde
dissimilis est. Sectio *Stenandrae*. (Fig. 6)

Perennis laxe caespitans, *rhizomate* breviuscule repente
estolonifero. *Culmi* erecti 4–5.5 cm alti graciles rigidi acute
triquetri striati supra medium scabri. *Folia* anguste linearia
rigida 1.5–2 mm lata culmum aequantia vel paullo superantia
plicata canaliculata margine scaberrima apice sensim attenuata
longe acuminata. *Vaginae* basilares fuscae fuscorubentesve
rigidae aphyllae inferiores subsquamiformes. *Spicula* solitaria
anguste linearia 5–7 cm longa 2 (–3) mm lata perlaxe florens apice
in *partem masculam* 10–15 (–20) mm longam vix attenuata,
rhachi saltem infra medium visibili. *Bractea* nulla. *Squama*
foeminea ovato-oblonga 5–6 mm longa rigida brunnea fuscave
partim castanea apice acultiuscula basi fere angustata utriculum
amplectens, *costa* latiuscula 1–3-nervosa resinosolineolata.
Utriculus erectus linearioblongus vel lanceolatus squamam
paullo superans 6–6.5 (–7) mm longus adpresse trigonus gla-
berrimus maturitate fuscatus tenuiter plurinervulosus basi sensim
attenuatus in *stipitem* longiusculum apice subsensim attenuatus
in *rostrum* breviusculum conicocylindricum, *ore* oblique secto
margine anguste albohyalino. *Nux* arcte inclusa linearioblonga
3–3.5 mm longa obtuse trigona apice basique subito contracta,
stylo brevi basi paullo incrassato subconico supra medium usque
trifido. *Racheola* setiformis utriculum duplo superans recta apice hamata. *Fila-*
menta filiformia elongata.

Fig. 6. *Uncinia Ohwiana*,
n. sp. a. spicula; b.
squama floris foeminei;
c. utriculus cum ra-
cheola et stigmatibus;
d. nux cum parte
racheolae (e typo).

N o v a-G u i n e a: sine loco speciali. TNS. no. 92552 !—holotypus.

Summary

In the present paper, five species and one variety of sedges, grass and pipewort were newly proposed and one sedge was reduced to a varietal rank. A special mention is necessary for a new sedge of *Garciles* from Java. So far as Thunberg's type, *Carex brunnea* is characterised by somewhat yellowish-green leaves and very small perigynia 2.5–2.7 mm long, hence Dr. Kükenthal once considered true *C. brunnea* to be a variety of *C. gentilis*. Although the species of *Graciles* have very wide deviation, the above plant from Java is not *C. brunnea*. Further, Malaysian plants treated so may possibly be quite different from *C. brunnea*. The abbreviations TI and TNS are used for the herbaria of the University of Tokyo and of the National Science Museum, Tokyo, respectively.

Physiological Changes in the Germinating Seeds during Low Temperature Treatment II
On the Activity of Catalase and Peroxidase

by Shôjirô Inoue* and Masaki Yahiro**

井上昭治郎・八尋正樹: 低温処理間の発芽種子の生理的変化 II
カタラーゼ及びペルオキシダーゼについて

Introduction

Many biennial plants, which initiate into the reproductive growth by the long day-length of spring, require chilling of a certain days in the early stage of seedling.

It is considered that the study of the physiological changes in the seedling during the progress of vernalization is a basic matter for dissolving the problem of the reproductive growth.

On the relation between the vernalization and the enzymes, Oveckin and others[1] made researches in the winter wheat, Sapoznikova[2] in *Lupinus angustifolius;* and B. Sen[3] investigated diastase and phosphatase in the wheat and lipase, catalase and phosphatase in the mustard and diastase in the barley.

In the present experiment the authors dealt with the determination of some enzyme activity, above all, catalase and peroxidase that may have a considerable connection with the respiration.

It is their great pleasure to record here a debt of gratitude to Professor Hitoshi Kojima for his very valuable advice during the work and the authors also wish to express their thanks to Professor Shirakawa for his very helpful suggestions.

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 * Botanical Laboratory, Faculty of Agriculture, Kyûshû University. 九州大学農学部植物学教室
** Sericultural Laboratory, Faculty of Agriculture, KagoshimaUniversity. 鹿児島大学農学部蚕蚕
　　学教室

Material and Methods

As the material the Japanese radish plant (*Raphanus sativus* L. var. *raphanistroides* Makino, race "Minowase") was used. The plant rapidly initiates reproductive growth by a low temperature treatment of 3 to 7 days at 2 to 10° C.

Seedings germinated by the usual method (1954), employed in the Botanical Laboratory, Faculty of Agriculture, Kyûshû University, were placed in a refrigerator of 5 to 7°C ("treated seedlings") or in an incubator at 30°C ("untreated seedlings") for a definite period of time.

Every day both treated and untreated seedlings were taken out from the refrigerator or incubator just before the experiment, and were determined of the activity of catalase and peroxidase. One gram of plumules which were thought to be the responsive part to the vernalization (1954) containing hypocotyls (except cotyledons) was ground down in the mortar with 7.5 ml of $\frac{1}{15}$ M-phosphate buffer solution and 17.5 ml of distilled water. This solution was used as the enzyme solution. For the determination of catalase activity Okuda's method[5] was employed and the value of K (which was shown by $\frac{k}{g}$ of fresh weight of plant) was calculated according to the following formula:

$$K = \frac{1}{t} \cdot \log_{10} \frac{a}{a-x},$$

where t is the time denoted in minutes, a and a−x are potassium permanganate in millilitres exhausted at 0 min. and t min., respectively.

Okuda's method was adapted also for measurement of peroxidase activity. Ten ml of peroxidase sample to be tested was mixed with 30 ml pyrogallol–H_2O_2 solution (0.1g pyrogallol plus 5 ml of 0.01N H_2O_2 and some distilled water), and the reaction was interrupted after 5 minutes by the addition of 2 ml. of 1N–H_2SO_4. Purpurogallin, which had been turned into yellow, was extracted with 20 ml of ether and was colorimetrically measured as compared with a standard solution.

Experimental results and discussion

1) Catalase activity

Seeds of the radish plants used in this experiment are easily effected by a low temperature treament of 5 to 7°C for 6 days and initiate flowering.

The changes in the catalase activity of both the treated and untreated seedlings are plotted in Fig. 1, where the catalase activity of the treated seedlings is always higher than that of the untreated ones.

Catalase activity of the material treated by low temperature for 6 days, at that time the effect of vernalization seems to complete, showed the lowest value of activity (Fig. 1), and no further lower activity could be recognized in the curve of Fig. 2.

Between the peroxidase activity, which will be shown later, and the catalase
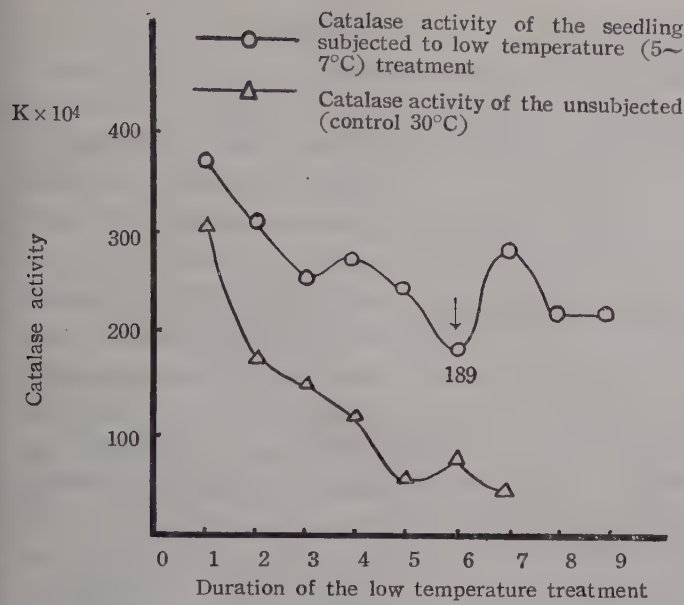
Fig 1. The change of catalase activity during the course of
vernalization
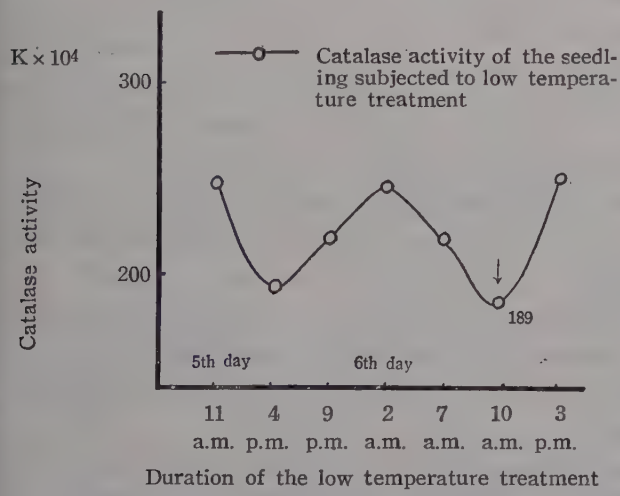
Fig. 2. The change of catalase activity from the 5th to 6th
day of vernalization

activity, there existed a reversal relationship in the process of the low temperature treatment.

Oveckin and others[1] Sapoznikova[2] and B. Sen [3] described that the activities of catalase and peroxidase are valuable for distinguishing vernalized seeds from unvernalized ones. They reported also that catalase activity of the treated seedling is always higher than that of the untreated one; this result is in accord with that of the present experiment.

2) Peroxidase activity

Some material used in this measurement was the seed, which could be vernalized by the low temperature treatment after about 7 th day from the beginning of the treatment.

Peroxidase activity of the treated seeedling was generally higher than that of the untreated one as seen in Fig. 3.

There was an ascent of the activity of peroxidase beginning from about the 6 th day of the low temperature treatment onwards; and it was thought that after that day the vernalization should be seemingly accomplished.

On the other hand, seeds that began to show the effect of vernalization after about 5th day of the low temperature treatment, showed a remarkable increase in the peroxidase activity after about 5th day of the treatment. It seems likely that there

is a close relation between peroxidase activity and vernalization.

The change in peroxidase activity of the treated seedling is more conspicuous than that in catalase activity of the same seedling. However, as is the case with catalase, the peroxidase activity of seedling subjected to low temperature is higher than that of the unsubjected seedling. Knox and others[6] studied on the relation between the catalase and the peroxidase but the problem is not yet completely elucidated.

Peroxidase activity of the seedling subjected to low temperature (4~7°C) treatment

Peroxidase activity of the unsubjected (control: 23~24°C)

Duration of the low temperature treatment

Fig. 3. The change of peroxidase activity during the course of vernalization

Summary

Activity of catalase and peroxidase in the seedling of radish plants (*Raphanus sativus* L. var. *raphanistroides* Makino, race "Minowase") during low temperature treatment was measured.

It was found that the activities of the catalase and the peroxidase in the seedling under the low temperature treatment are always higher than those in the untreated seedling.

But the catalase activity falls gradually with the progress of time in both the seedling subjected to the low temperature treatment and the unsubjected.

On the other hand, the peroxidase activity rises sharply on the day when the effect of vernalization begins to appear.

It may be assumed that the rise in peroxidase activity on the day, when the low temperature treatment takes place, has some relation to the vernalization.

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On the Ecological Meaning of Transparency for the Production of Matter in Phytoplankton Community of Lake

by Shun-ei ICHIMURA*

市村俊英: 湖沼植物プランクトン群落の物質生産における透明度の生態的意義について

The turbidity in natural waters as estimated by transparency has been used empirically as an indicator for the comparison of productivity among various waters. Surveys dealing with transparency have been reported by many investigators. Such works, however, have not sufficiently been made from the ecological point of view. Consequently, the biological informations concerned with the meaning of transparency for the matter production by green plant are scanty at present. The turbidity plays a leading role in determining the quantity of light transmission in the lake, so that the general effect of turbidity on the aquatic plant manifests itself in the restriction of photosynthetic process on which the production of organic matter in lake depends. From this point of view, the basic clue to analysis of the ecological meaning of transparency for the biological production may be found in the relationship between the photosynthesis and the intensity of illumination. The present paper deals with studies on the ecological meaning of transparency for the production of matter in phytoplankton community of lake.

Transparency and its relation to the light penetration in the lake

It has well been known that the light intensity in lake can be expressed by Lamberr-Beer's law:

$$I_x = I_0 e^{-\alpha x} \quad \cdots \cdots \cdots (1)$$

in which I_0 is the initial light intensity, I_x the light intensity at water depth (x), and α the extinction coefficient of lake water, respectively. Kikuchi (1935) recognized the following relation between the transparency (t) and the extinction coefficient (α) of lake water:

$$\alpha = \frac{1.9}{t} \quad \cdots \cdots \cdots \cdots \cdots (2)$$

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* Botanical Institute, Faculty of Science, Tokyo University of Education. 東京教育大学理学部
植物学教室
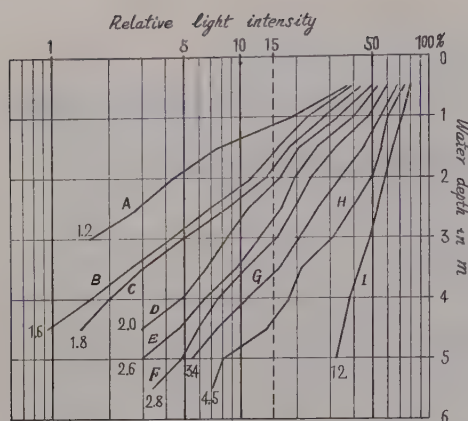
Fig. 1. Transparency and light intensity in lakes.

The figures on the curves indicate the depth of transparency.
A: Sugao, June (1951) B: Suwa, October (1949) C: Nakanuma, June (1950) D: Suwa, July (1949) E: Nakanuma, December (1950) F: Nakanuma, September(1950) G: Yamanaka, September (1950) H: Tatenoumi, December (1948) I :Motosu, November, (1949)
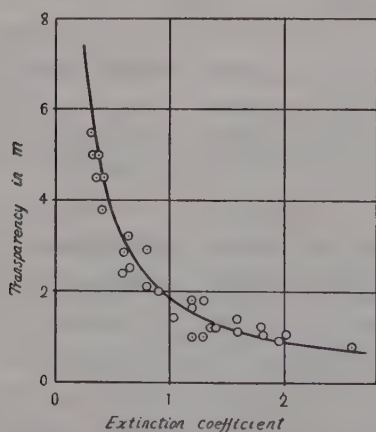
Fig. 2. Relation between transparency and extinction coefficient in lakes.

In order to confirm Kikuchi's result, the author measured the transparency and the light penetration at several lakes of central Japan. Transparency was determined with Secchi's disc, and the light penetration with electric photometer (Toshiba). The results are summarized in Fig. 1. The light intensity diminished with increasing depth and reached at the depth of transparency 12 to 18 % (mean value was ca. 15 %) of the surface light intensity. From these observations, 15 % may be allowed as a general value at the depth of transparency in lake. If $\frac{It}{I_o}=0.15$, then $\alpha=\frac{1.9}{t}$ which conforms with the equation (2). The relation between transparency and extinction coefficient measured at several lakes is shown in Fig. 2. As pointed out by Hanaoka (1955), the values of light intensity at the depth of transparency in various waters do not always coincide each other. If above relation can be regarded as a general rule, at least in lakes, α can be given from the transparency by the equation (2) and putting the value into the equation (1), we shall get the light intensities at different depths of lake with the transparency. Therefore, we can obtain the light intensity in lake indirectly by the measurement of transparency.

Relation between the transparency and the standing crop of phytoplankton

The transparency is determined by the quantity of seston and shows considerable variation with the seasonal change. Several workers have already examined the relation between the transparency and the suspended matter in lake [Yoshimura (1937), Chandler (1942), Hogetsu et al. (1952), Verduin (1954)], and it has been shown that there is an inverse relation between them. The author made some researches on

the effect of the amount of seston upon the transparency in lake. The measuring method of seston was the same as employed in the previous paper (Hogetsu and Ichimura 1954). The results are illustrated in Fig. 3, in which curvilinear relationship between the amount of seston and the transparecy can be recognized. As the seston consists of two components [Bioseston and Abioseston–Welch, (1952)], the results do not indicate exactly enough the amount of phytoplankton. For this reason the chlorophyll content in the unit volume of lake water containing phytoplankton has recently been determined for the quantitative measurement of phytoplankton.

Fig.4. Relation between transparency and amount of seston in lakes.

The author (1956) suggested an inverse relation to exist between the transparency and the chlorophll content of eutrophic lake. The amount of phytoplankton can be approximately given from that of chlorophyll in water [Atkins et al. 1953, Hogetsu and Ichimura (1954)], so that the quantitative relation may also be presumed between the standing crop of phytoplankton and the transparency. As can be seen in Fig. 4, the relation between them, which was calculated from the data in previous paper [Ichimura (1956), p. 9], can be given by following equation:

$$\log y = -1.493 \log t - 0.053 \cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots (3)$$

in which y (mg/l) is the mean value of standing crop of phytoplankton in epilimnion. Of course, the equation (3) is not always fitted to every water. However, as far as the eutrophic or the mesotrophic lake is concerned, this equation may hold good under normal conditions of the lake.

Determination of trophogenous layer from transparency

As the underlimit of trophogenous layer is restricted by the compensation depth, so the determination of the compensation depth becomes important for the study of the production of lake. Results of laboratory experiments carried out by many investigators on the photosynthesis of algae permitted to give sufficient, though approximate, information on the light intensity at the compensation point of algae. Generally it is agreed that the maximum rate of photosynthesis is found at the light intensity of 50 kilo-lux and that the compensation point lies at ca. 500 to 600 lux in green algae and 400 lux in diatoms. Therefore, the light intensity at the compensation point is about 1 to 2% of that at which maximum photosynthesis is obtained. The mean daylight intensity at noon is assessed as ca. 50 kilo-lux on a
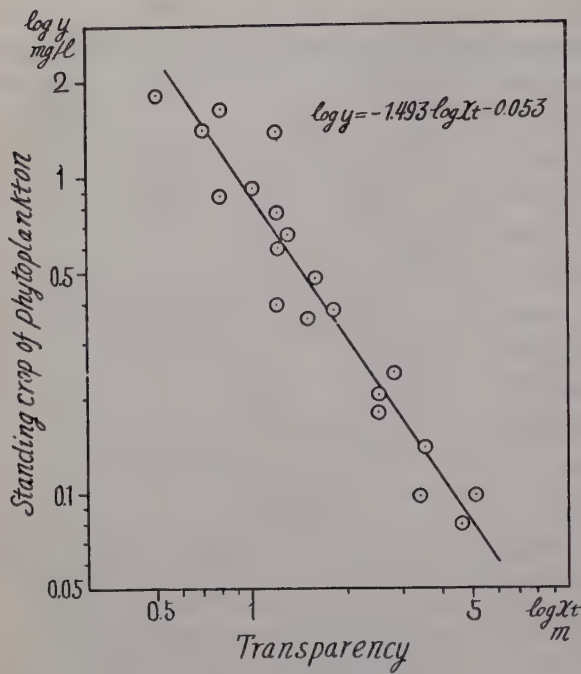
Fig. 4. Relation between transparency and standing crop of phytoplankton in some eutrophic lakes.

fine day and 10 kilo-lux on a cloudy day in Japan. If 500 lux is assumed to be the compensation light intensity of phytoplankton, the light penetrating to the compensation depth may range between 1 and 5%. From the equation (1) and (2), therefore, the depth of compensation (x_c) is:

Fine day　　$x_c=2.4$ t······(4)

Cloudy day　$x_c=1.6$ t······(5)

Naturally, the vertical shift of compensation depth due to diurnal and seasonal change of sunlight occurs. Further, it must be noticed that the respiration of plankton measured in the field include not only that of phytoplankton in

day and nighttime but also that of zooplankton and other microorganismus, whereas in the laboratory measurements these effects are eliminated. Consequently the light intensity at the compensation depth in lake may be somewhat greater than that

Table. 1. Relation between transparency and compensation depth in lakes.

Transparency m	Compensation m	Comp./Trans.	Light intensity % at compensation
1.6	4.1	2.6	2,7
1.8	4.2	2.3	3.0
2.0	4.2	2.1	2.5
2.2	4.5	2.0	3.6
2.4	4.5	1.9	4.8
2.8	5.0	2.0	4.0
3.4	7.7(?)	2.3	2.4(?)
Average		2.2	3.3

measured in laboratory (Table 1). According to Welch (1952) and Clark (1954), the alternation in spectral composition at different depth is affected very widely by the turbidity and it has well been known that the compensation point is determined not only by light intensity but also by wave length. Therefore, it appears reasonable to expect that departure from the values given by these equations may occur in

some circumstances. As we had already examined the equation (2), however, it will be said, at least on the eutrophic or the mesotrophic lake, that the compensation depth of phytoplankton or the trophogenous layer is about 2.4 times on a fine day and 1.6 times on a cloudy day as large as the depth of transparency. These results had already been recognized practically by some investigators. [Hogetsu (1939a), (1939b), Clarke (1946),
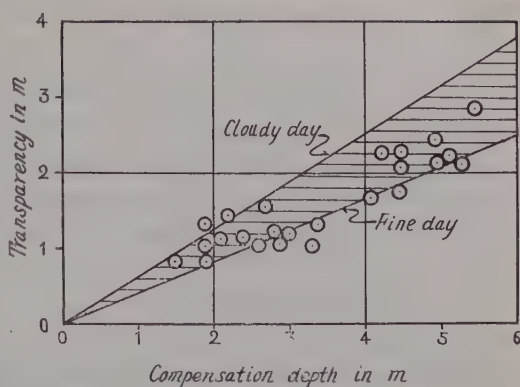
Fig. 5. Relation between transparency and compensation depth in some eutrophic lakes.

Hogetsu and Ichimura (1954)]. The additional evidences in support of this consideration were also obtained by the author (Fig. 5).

Effect of turbidity upon the production in phytoplankton community of lake

Mean hourly assimilation curve of phytoplankton is represented by the following equation [c.f. Tamiya (1951)].

$$q=\frac{bI}{1+aI} - r \cdots\cdots\cdots\cdots\cdots(6)$$

where, q is the net assimilation, a and b are constant, I the light intensity and r the respiration. Daily assimilation curve can be constructed by the combination of an hourly assimilation curve of phytoplankton and the mean daily march of illumination [Monsi and Saeki (1953), Kasanaga and Monsi (1954)].

Assuming that the photosynthesis of phytoplankton does not suffer "the surface inhibition", the daily net assimilation (q) of unit amount of phytoplankton at depth of x may be written from the equation (1), (2) and (4), as follows:

$$q=\frac{bI_oe^{-\frac{1.9}{t}x}}{1+aI_oe^{-\frac{1.9}{t}x}} - r \cdots\cdots\cdots\cdots(7)$$

The total daily net production in a column of unit area extending from the surface of water to the depth(x) is given by integrating the equation (7),

$$P=\frac{yxb}{1.9a}\left(2.303\log\frac{1+aI_o}{1+aI_oe^{-\frac{1.9}{t}x}}\right)-yxr\cdots\cdots\cdots(8)$$

where p is the total net production per unit area of lake surface, y the amount of phytoplankton in unit volume of lake water. This equation is introduced with an assumption that the phytoplankter distributes homogeneously and has same photo-synthetic activity. If vertical circulation of water is so rapid that each phytoplankter is illuminated equally, the light intensity in equation(8) can be represented in epilimnion simply by the average light intensity. From the equation(3) the standing crop of phytoplankton is re-written as a function of transparency.

$$y = 0.89t^{-1.49} \cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots (9)$$

So that the quantum yield y(mg/1) may be evaluated from measurements of trans-parency. Equation (7) can now be stated:

$$P = 0.89t^{-1.49} \cdot \frac{x \ b}{1.9 \ a}\left(2.303 \log\frac{1+aI_o}{1+aI_o e^{-\frac{1.9}{t}x}}\right) - 0.89 \ t^{-1.49} \cdot xr \cdots\cdots (9)$$

The influence of turbidity on the production of phytoplankton may be evaluated by these mathema-tical treatment. As an example of such calculation the results of the measurements made on a partly cloudy day in summer are shown in Fig. 6, which indicates the relation between the depth of lake and the net production under the various transparency. From Fig. 6, it will become clear that total net production per unit area increases with increasing depth of lake within the limits of the trophogenous layer, and reaches its maximum, when the depth of lake accords with that of compensation of phyto-plankton, and then diminishes with further increase of depth.

Furthermore, the effect of turbidity upon the production is greater within the range of high turbidity than the low turbidity. Namely, it may be said that the total net production of lake is determined by the volume relations between the epilimnion and hypolimnion under a definite trans-parency. As shown in the foregoing consideration, the light intensity at the compensation depth

Fig. 6. Relation between the depth of lake and net production of matter in phytoplankton community of lake with various transparency.

approximately ranges between 1 and 5 % of that of surface. If it proves to be adequate, the maximum production will be calculated immediately by replacing x by (4) or (5) in the equation (10).
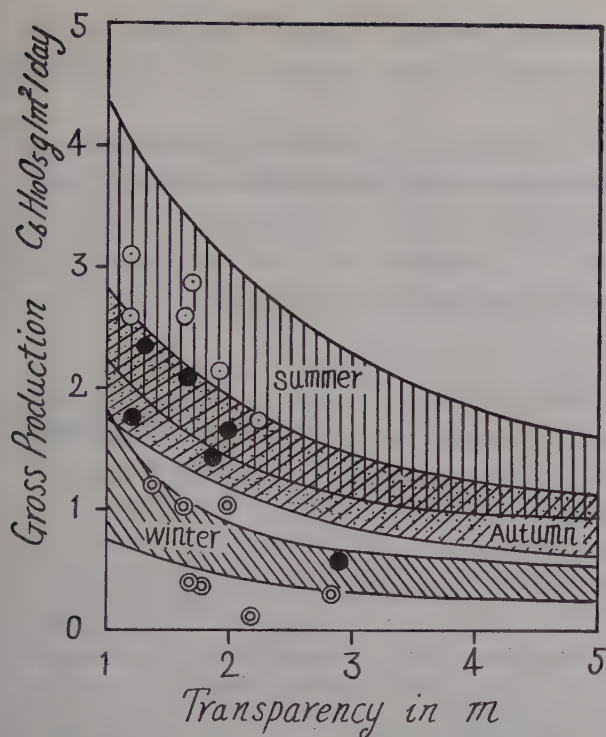
Fig. 7. Gross production of dry matter in phyto plankton community of lakes with various transparency. Limits of seasonal productions were obtained by theoretical calculations and the plots by observations in the field. (⊙ = summer, ● = autumn, ◎ = winter)

The amount of feeding or of respiration of consumer (fish, zooplankton etc.) and of decomposer (bacteria) are neglected in this equation. According to Hogetsu and Ichimura (1954), Ichimura (1954), the amount of dissolved oxygen which is consumed by respiration of the consumer is approximately 33—120 % of the gross production of phytoplankton. Therefore, it may be expected in the application of this equation to natural waters that the calculated values in net production are much higher than that of observed. However, the calculated values in gross production of phytoplankton community were approximately in accord with that of direct observation in the field.

The method of direct measurement had been fully described in the previous paper [Hogetsu and Ichimura (1954)]. Fig. 7 indicates the comparison of the calculated with the observed gross production. The agreement of these values indicates clearly that we can quantitatively compare the productive capacity of phytoplankton communities among various lakes on the basis of the transparency.

Summary

The present studies are undertaken to obtain an ecological information concerning the effect of transparency upon the matter production in phytoplankton community of lake and it is demonstrated both experimentally and theoretically that the transparency can be used as an indicator of the productivity of lake.

1) Under the normal condition, the relation between the amount of phytoplankton and the transparency is expressed quantitatively by a simple equation(3).

2) The light penetrating in any depth is estimated approximately from the transparency by Beer's equation and the depth of trophogenous layer is about 2.0 times as large as that of transparency.

3) The effect of turbidity upon the matter production of phytoplankton is assessed by mathematical analysis in which the rate of production is combined with

a daily assimilation curve of phytoplankton and daily march of the intensity of illumination in waters. The good agreements are found between the calculated and observed values.

   4) The effect of turbidity upon the production of matter in phytoplankton community is more remarkable within the limits of the high turbidity than in the case of the low turbidity.

   5) From the above considerations, it becomes clear that the amount of the production of matter in phytoplankton community can be estimated indirectly from the transparency.

The author wishes to express his thanks to Prof. M. Monsi and Prof. K. Hogetsu, under whose guidance this research has been carried out. The author also wishes to acknowledge with thanks the encouragement and advice of Prof. T. Miwa.

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本　会　記　事

支 部 通 信

中部支部

　第4回講演大会（5月6日，於愛知学芸大，生物学教室）大原準之助，西三河の Flora について。浜島繁隆・森隆也，花粉の貯蔵と呼吸について。渡辺祐二，森隆也，ナタネ科植物の花粉の人工発芽について。高木典雄，中部高山地帯ヒツジゴケ科蘚類について。川松重信・加藤勉・伊藤進・近藤静代，高等植物数種 の 原形質連絡の観察。高尾昭夫，クロマツ受精前後における Millon 反応について。谷口森俊，三浦半島の落葉樹林。矢頭献一，紀伊半島に於けるヤマトタチバナの分布。加藤幸雄，シダ仮根の分化と伸長に関する新知見。熊沢正夫，いわゆるコクサギ型葉序に就いて。沢井輝男，酵母菌のアミラーゼ——アミロ・トランスグルコシダーゼ。岡田善敏，珍らしい竹の変種について。瀬木紀男，ヒトエグサ（緑藻）に就いて。倉内一二，海岸林と塩風害。

Studien über Anthocyane, XXVIII.
Papierchromatographische Übersicht der Anthocyane im Pflanzenreich (III). Über Anthocyane der Gebirgspflanzen

von Kôzô HAYASHI und Yukihide ABE*

林 孝三 ・ 阿部幸顕： アントチアン色素の研究 （第28報） 高山植物の色素

Seitdem die von Robinson und Robinson (1) eingeführte Schnellmethode zur Identifizierung der Anthocyane in den Pflanzenextrakten zur Untersuchung von zahlreichen Wild und Kulturpflanzen herangezogen wurde, sind mehrere Arbeiten in verschiedenen Ländern erschienen, die zur Erweiterung unserer Kenntnis über die Verbreitung der Anthocyane in der Natur erheblich beigetragen haben. So sind eine Reihe von Arbeiten von Robinson und seinen Schülern (1,2) in England, eine Arbeit von Gascoigne *et al.* (3) in Australien, ferner Arbeiten von Forsyth und Simonds (4) in Trinidad (B. W. I.), und von Hayashi und Abe (5) in Japan zu nennen. In allen bisherigen Arbeiten bezogen sich die Versuchsobjekte hauptsächlich auf Pflanzen der Ebene, sodass unsere Kenntnis über die Anthocyanfarben zahlreicher Gebirgs-pflanzen heute noch recht mangelhaft ist. Um diese Lücke auszufüllen, haben wir uns mit diesen Pflanzen befasst.

Material und Methode

Der vorliegende vorläufige Bericht umfasst 105 Pflanzen aus 34 Familien, die in Gebirgen von Mitteljapan einheimisch sind. Beim Einsammeln des Versuchsmaterials hat uns Herr S. Nakamura, Obergärtner im Botanischen Garten in Nikko, vielseitig unterstützt, wofür wir ihm zu grossem Dank verpflichtet sind. Trotz unserer Bemühungen ist unsere Arbeit noch unvollständig und muss in der Zukunft an Hand weiterer Pflanzenobjekte ergänzt werden, bevor wir die Verbreitung der Anthocyane in der Alpenflora Japans ausführlich kennen lernen können.

Die Aufarbeitung zur Charakterisierung des Anthocyans verlief im grossen und ganzen nach der bereits angegebenen, papierchromatographischen Technik (6). Hierzu wurden frische Blüten bzw. Früchte mit kalter 1-proz. methanolischer Salzsäure extra-hiert, und die erhaltene rote Anthoacynlösung wurde auf die Startlinie eines Filtrier-papiers (Tôyô Nr. 50, 40×40 cm) gestrichen. Unter Benutzung von einem der unten

1) XXVII. Mitteil : Bot. Mag. Tokyo 68: 299 (1955).

beschriebenen Lösungsmittelgemischen wurde danach ein Bandchromatogramm zur Entwicklung gebracht. Darauf wurde jede der entstandenen Farbzonen sorgfältig ausgeschnitten und gesondert mit essigsäure-haltigem Methanol auf übliche Weise eluiert (hierzu vgl. Literatur (7)). Dieses Eluat wurde nach dem Einengen aufs neue zur exakten chromatographischen Analyse gebraucht. Dadurch liess sich die Glykosidstufe des Anthocyanins auf dem Chromatogramm gut nachweisen (8). Immerhin ist es auch sehr behilflich, den Verlauf der milden Hydrolyse chromatographisch näher zu verfolgen, wobei das Anthocyanin in der Regel zur Bildung von den niedrig glykosidifizierten Produkten stufenweise abgebaut wird. An jedem der Hauptbestandteile des Blütenanthocyanins haben wir dieselbe Methode zur Bestimmung von Glykosidstufen benutzt. Bei dieser Untersuchung haben sich die folgenden Lösungsmittelgemische als gut bewährt:

Essigsäure/konz. Salzsäure/Wasser (5: 1: 5 v/v) .... für Aglykon,
              „   (3: 1: 8. v/v) .... für Glykosid,
iso-Amylalkohol/konz. Salzsäure/Wasser (5:1: 1, v/v). .... für Aglykon
                                                                    und Glykosid,
n-Butanol/Essigsäure/Wasser (4: 1: 5, v/v) .... für Glykosid,
n-Butanol/konz. Salzäure/Wasser (7: 2: 5, v/v)........................ für   „ ,
Phenol/Wasser (9: 1: in Gew.) .... für methoxyl-haltiges Glykosid.

Auf jeden Fall ist es unbedingt nötig, die Bestimmung des Aglykons genauer zu führen. Hierzu arbeitet man gewöhnlich auf folgende Weise: Das obige methanolisch-essigsäure Anthocyaneluat wird im Vakuum unter gelindem Erwärmen abgedampft, der Rückstand in ein wenig 20-proz. Salzsäure aufgenommen, und durch kurzes Kochen (2 Min.) vollständig hydrolysiert. Nach dem Verdünnen mit etwas Wasser wird das entstandene Aglykon mit ein wenig iso-Amylalkohol entzogen, und die Amylalkoholphase sodann auf chromatographisches Papier beschickt. Um die Identifizierung der einzelnen Aglykone einwandfrei zu erzielen, empfiehlt es sich, die Lösungen einiger authentischer Anthocyanidine als Kontrolle auf demselben Papier nebenbei tropfenweise hinzuzufügen.

Auf diese Weise konnten wir sowie die Anthocyanidine als auch die Glykosidstufen der betreffenden Anthocyanine gut nachweisen. Hierbei wurde aber die Bestimmung der Zuckerkomponenten des Glykosids ausser Acht gelassen, weil die verfügbare Materialmenge meistens sehr gering war, was die Aufarbeitung nahezu unmöglich machte.

Versuchsergebnisse

Der Übersichtlichkeit halber wurden die gewonnenen Daten in der untenstehenden Tabelle zusammengestellt, wobei die Versuchspflanzen nach dem natürlichen Pflanzensystem Englers angeordnet wurden. In der Tabelle haben wir auch die Vegetationszone jeder Pflanze mit der Bezeichnung A, B, C und D angegeben, wobei uns Herr Dr. H. Itô behilflich war.

Weiterhin sind die in der unten stehenden Tabelle angegebenen Bezeichnungen derart zu verstehen, dass sich grosse Buchstaben auf die Hauptverbreitung und kleine auf das Vorkommen in Minderzahl der betreffenden Pflanzen in den genannten Regionen beziehen.

Zieht man nun die Anthocyanidintypen in Betracht, so lassen sich unsere Daten, insofern es sich um den Blütenfarbstoff handelt, folgendermassen zusammenfassen:

A: alpine Region   (gegen 2400–2500 M. üb. d. Meeresspiegel),
B: subalpine Region   (um 1500–1700 M.   „   ),
C: montane Region   (um 1000–1300 M.   „   ),
D: basale Region   (unterhalb 1000 M.   „   ).

Anthocyanidintypen* / Vegetationszone	Pelargonidintypus	Cyanidintypus	Delphinidintypus	Summe
A**	0	6	4	10
AB	1	3	4	8
B	0	1	3	4
BC	0	12	3	15
C....................	1	5	7	13
BCD....................	0	2	0	2
CD	1	7	6	14
D....................	1	7	8	16
Summe	4	43	35	82

* Bezogen auf den Hauptanthocyanidin-Bestandteil.
** Die Abkürzungen sind wie folgt zu verstehen: A umfasst A und Ab; AB······AB und ABc; B······aB, B und Bc; BC······aBC, BC und BCd; C······bC, C, bCd und Cd; BCD······BCD; CD···· ·bCD und CD; D······cD und D.

Freilich können wir hieraus nichts Bestimmtes aussagen. Wenn man aber jede dervers chiedenen Vegetationszonen eingehend prüft, so wird man bald darauf aufmerksam gemacht, dass je höher man steigt umso mehr findet man violett- bis blau-blühende Pflanzenarten, die Delphinidinfarbstoffe fürhen müssen, wie auch von Th. Weevers (9) betont wurde. Dies führt zu der interessanten Aufgabe, die obwaltenden Beziehungen zwischen den Oxyationsstufen des Anthocyans und den extremen Umweltbedingungen in den Gebirgen, z.B. intensives Ultraviolett und Kälte, von ökologischem Gesichtspunkte aus eingehend zu analysieren.

Literatur

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Tabellarische Zusammenstellung der Anthocyane

Nr.	Familien	Versuchspflanzen[1]		Pflanzenteil	Farbe
1	Pinaceae	*Pinus pumila* Regel	ハイマツ	weibl. Blüten	rotbraun
2	Polygonaceae	*Tovara filiformis* Thunb.	ミズヒキ	Petale	rot
3	Caryophyllaceae	*Dianthus superbus* L. var. *speciosus* Reichb.	タカネナデシコ	"	"
4		*Lychnis fulgens* Fischer	エゾセンノウ	"	scharlachrot
5		*L. miqueliana* Rohrb.	フシグロセンノウ	"	"
6		*Melandryum keiskei* Ohwi	オオビランジ	"	schwach rotpurpur
7	Ranunculaceae	*Aconitum japonicum* Thunb.	トリカブト	"	blauviolett
8		*Anemone nipponica* Merr.	シュウメイギク	"	schwach rotviolett
9		*Anemonopsis macrophylla* Sieb. et Zucc.	レンゲショウマ	"	rotpurpur~violett
10		*Aquilegia buergeriana* Sieb. et Zucc.	ヤマオダマキ	Sepale	bräunlich purpur
11		*Clematis stans* Sieb. et Zucc.	クサボタン	Petale	schwach blauviolett
12		*Thalictrum rochebrunianum* Franch. et Sav.	シキンカラマツ	"	schwach violett
13	Berberidaceae	*Diphylleia grayi* Fr. Schm.	サンカヨウ	Fruchthaut	blauviolett
14	Saxifragaceae	*Hydrangea serrata* Seringe	ヤマアジサイ	Schaublüten	"
15	Rosaceae	*Filipendula multijuga* Maxim.	シモツケソウ	Blüten	rot
16		*Prunus maximowiczii* Rupr.	ミヤマザクラ	Frucht	dunkel rot
17		*P. nipponica* Matsum.	ミネザクラ	"	"
18		*P. sargentii* Rehder	オオヤマザクラ	Petale	rosa
19		*Rosa acicularis* Lindl.	オオタカネイバラ	"	"
20		*R. acicularis* Lindl. var. *nipponensis* Koehne	タカネイバラ	"	"
21		*R. marretii* Lév.	カラフトイバラ	Frucht	rot
22		*R. rugosa* Thunb.	ハマナス	Petale	"
23		*Rubus buergeri* Miquel	フユイチゴ	Frucht	scharlachrot
24		*R. mesogaeus* Focke	クロイチゴ	"	dunkel rot
25		*R. pectinellus* Maxim.	コバノフユイチゴ	"	rot
26		*R. vernus* Focke	ベニバナイチゴ	"	"
27		*Sanguisorba hakusanensis* Makino	カライトソウ	Blüten	rotviolett
28		*S. carnea*	ワレモコウ	"	rot
29		*Sieversia pentapetala* Greene	チングルマ	Achänen-haare	rotbraun
30		*Spiraea salicifolia* Linn.	ホザキシモツケ	Blüten	rot
31	Leguminosae	*Astragalus adsurgens* Pall.	ムラサキモメンヅル	Petale	rotpurpur
32		*Trifolium lupineaster* Linn.	シャジクソウ	"	schwach rotpurpur
33		*Vicia nipponica* Matsum.	ヨツバハギ	"	violett
34	Geraniaceae	*Geranium erianthum* DC.	チシマフウロ	"	blauviolett

1) Lateinische Pflanzennamen wurden von Herrn Dr. H. Itô durchgesehen, wofür wir ihm bestens danken.

2) Einzelne Anthocyanidine wurden nach der sauren Hydrolyse des ursprünglichen Pflanzenextraktes sogleich auf papierchromatographischem Wege nachgewiesen.

in den Gebirgspflanzen in Japan.

Anthocyanidin-Bestandteile[2]	Anthocyaninkomponente[4]	Lokalisation d. Pflanze[5]	Nr.
Cya[3] (10)[4]	Cyanidin-gylcosid (3-Monohexosid?)	A	1
Cya (10)	Cyanidin-3-monosid (Chrysanthemin?) (5) +3-hexopentosid (Keracyanin?) (5)	D	2
Cya (10)	Cyanidin-3·5-dihexosid (10)+Cya-3-monohexosid (Spur)	A	3
Pel (10)	Pelargonidin-glycosid	C	4
Pel (10)	Pelargonidin-3-monohexosid (7)+3-hexopentosid (3)	CD	5
Cya (10)	Cyanidin-glycosid (in 3 Formen)	C	6
Del (10)	Delphinidin-glycoside (in 2 Formen)	bCd	7
Cya (10)	Cyanidin-glycoside (in 4 Formen)	D	8
Cya (10)	Cyanidin-3-monohexosid (Chrysanthemin?) (7) +3-hexopentosid (Keracyanin?) (3)	bCd	9
Del(7)+Cya(3)	Delphinidin-glycoside (in 2 Formen)+5 unbekannte Flecken (Spur)	BCd	10
Del (10)	Delphinidin-glycoside (in 2 Formen)+unbekannt (Spur)	cD	11
Cya (10)	Cyanidin-glycoside (in 3 Formen)	CD	12
Cya (10)	Cyanidin-3-hexopentosid (10)+3-monohexosid (Spur)	B	13
Del (10)	Delphinidin-3-monohexosid (10)	CD	14
Cya (10)	Cyanidin-3-monohexosid (6)+3·5-dihexosid (4)	BCD	15
Cya (10)	Chrysanthemin (6)+Keracyanin (4)	bCd	16
Cya (10)	" (5)+ " (5)	aBC	17
Cya (10)	Cyanidin-glycoside (in 2 Formen)	Cd	18
Cya(8)+Päo(2)	Cyanidin-diglycosid (8)+Päonidin-diglycosid (2)	A	19
Cya (10)	Cyanidin-dihexosid (10)+3-monohexosid (±)	aB	20
Cya (10)	Cyanidin-glycosid + Leuco-cyanidin (haupt)	B	21
Cya(5)+Päo(5)	Cyanidin-glycosid (5)+Päonidin-glycosid (5)	BC	22
Pel(10)+Cya(±)	Pelargonidin-glycosid (10)	CD	23
Cya (10)	Chrysanthemin (7)+Keracyanin? (3)	CD	24
Pel (10)	Pelargonidin-3-monohexosid (10)+Cyanidin-glycosid (Spur)	CD	25
Cya (10)	Cyanidin-3-hexopentosid (Keracyanin?) (10)	AB	26
Cya (10)	Cyanidin-dihexosid (10)+3-monoglycosid (Spur)	A	27
Cya (10)	Cyanidin-3-monohexosid (Chrysanthemin?)+3andere Glycoside	CD	28
Cya (10)	Cyanidin-3-monohexosid (10)	A	29
Cya (10)	Cyanidin-3-monohexosid (8)+3·5-dihexosid (2)	C	30
Del(haupt)+Cya	——————————————	A	31
Mal (7)+Del(3)	Malvidin-3-monohexosid (10)	bCD	32
Mal+Del(±)	Malvidin-glycosid (10)	CD	33
Mal+Del(±)	Malvidin-glycosid (9)+unbekannt (1)	A	34

3) Abkürzungen: Pel=Pelargonidin, Cya=Cyanidin, Päo=Päonidin, Del=Delphinidin, Mal=Malvidin.

4) Ziffern in Klammern zeigen die scheinbaren Mengenverhältnisse der Farbstoffe.

5) Die Lokalisation der Pflanze wurde von Herrn Dr. H. Itô angegeben. Bezeichnungen: siehe S. 229.

Nr.	Familien	Versuchspflanzen	Pflanzenteil	Farbe
35		*Geranium eriostemon* Fisch. 　　var. *reinii* Maxim.　　グンナイフウロ	"	purpurrot
36		*G.*　　*yesoense* Franch. et Sav. 　　var. *nipponicum* Nakai　　アカヌマフウロ	"	"
37	Empetraceae	*Empetrum nigrum* L. var. *japonicum* K. Koch 　　　　　　　　　　　ガンコウラン	Frucht	schwarzrot
38	Aquifoliaceae	*Ilex sugeroki* Maxim. 　　var. *brevipedunculata* Ohwi　アカミノイヌツゲ	"	rot
39	Balsaminaceae	*Impatiens textori* Miq.　　　　ツリフネソウ	Petale	purpurrot
40	Lythraceae	*Lythrum salicaria* Linn.　　　　エゾミソハギ	"	"
41	Oenotheraceae	*Chamaenerion angustifolium* Scopoli　ヤナギラン	"	rotpurpur
42		*Epilobium pyrricholophum* Franch. et Sav. 　　　　　　　　　　　アカバナ	"	"
43	Araliaceae	*Oplopanax japonicus* Nakai　　　ハリブキ	Frucht	rot
44		*Panax japonicus* C. A. Meyer　　トチバニンジン	"	"
45	Cornaceae	*Chamaepericlymenum canadense* 　　Ascherson et Graebner　　ゴゼンタチバナ	"	"
46	Diapensiaceae	*Schizocodon soldanelloides* Sieb. et Zucc. 　　　　　　　　　　　イワカガミ	Petale	hellrot
47	Ericaceae	*Gaultheria adenothrix* Maxim.　　アカモノ	Frucht	scharlachrot
48		*Menziesia ciliicalyx* Maxim.　　ツリガネツツジ	Petale	rot
49		*Rhododendron kiusianum* Makino　ミヤマキリシマ	"	violettrot
50		*R. japonicum* Suringer　　　　レンゲツツジ	"	rot
51		*R. degronianum* Carr.　　　　　シャクナゲ	"	hellrot
52		*Tripetaleia paniculata* Sieb. et Zucc.　ホツツジ	"	"
53		*Vaccinium vitis-idaea* Linn.　　コケモモ	Frucht	rot
54	Primulaceae	*Primula cuneifolia* Ledeb. 　　var. *hakusanensis* Makino　ハクサンコザクラ	Petale	purpurrot
55	Oleaceae	*Ligustrum obtusifolium* Sieb. et Zucc.　イボタノキ	Frucht	schwarzviolett
56	Loganiaceae	*Buddleja japonica* Hemsl.　　　フジウツギ	Petale	hell purpurrot
57	Gentianaceae	*Gentiana makinoi* Kusnezow.	"	blauviolett
58		*G. scabra* Bunge var. *buergeri* Maxim.　リンドウ	"	"
59		*G. triflora* Pall. var. *japonica* Hara　エゾリンドウ	"	"
60	Boraginaceae	*Myosotis sylvatica* Hoffmann　　エゾムラサキ	Petale	hell bläulich
61	Verbenaceae	*Callicarpa japonica* Thunb.　　ムラサキシキブ	Frucht	hell violett
62		*C. mollis* Sieb. et Zucc.　　　ヤブムラサキ	"	"
63	Labiatae	*Chelonopsis yagiharana* Hisauchi et Matsuno 　　　　　　　　　　アシタカジャコウソウ	Blüten	rotpurpur
64		*Clinopodium chinense* O. Kuntze　　クルマバナ	"	"
65		*Prunella prunelliformis* Makino 　　　　　　　　　　タテヤマウツボグサ	"	violettrot
66		*P. vulgaris* Linn.　　　　　　ウツボグサ	"	"
67		*Salvia japonica* Thunb.　　アキノタムラソウ	Petale	blauviolett
68		*Scutellaria laeteviolacea* Koidz.　シソバタツナミ	"	violett
69		*Stachys japonica* Miq. var. *intermedia* Ohwi 　　　　　　　　　　イヌゴマ	"	schwach rotviolett
70	Scrophulariaceae	*Pedicularis chamissonis* Steven 　　var. *japonica* Maxim.　　ヨツバシオガマ	"	rotviolett
71		*P. resupinata* Linn.　　　　シオガマギク	"	"

Anthocyanidin-Bestandteil	Anthocyaninkomponente	Lokalisation d. Pflanze	Nr.
Mal (*10*)	Malvidin-diglycosid (*10*)+2 unbek. Flecken (*Spur*)	AB	35
Mal (*10*)	*"*　　　　(*10*)+ anderes Mal-glycosid (*Spur*)	BC	36
Del(*6*)+Mal(*4*)+Cya (±)	Empetrin (*6*)+ Uliginosin (*4*)	A	37
Cya (*10*)	Cyanidin-glycosid (3-hexopentosid?) (*10*)	A	38
Mal (*10*)	Malvidin-glycoside (in 4 Formen)	cD	39
Mal (*10*)	Malvin (*9*)+ Malvidin-3-monoglycosid (*1*)	D	40
Mal (*10*)	Malvidin-dihexosid (Malvin?) (*10*)+anderes Glycosid (*Spur*)	BC	41
Cya (*10*)	Cyanidin-glycosid	cD	42
Cya (*10*)	Cyanidin-glycosid (Lycoricyanin?)	A	43
Pel (*10*)	Pelargonidin-diglycosid (*8*)+ 3-monoglycosid (*2*)	cD	44
Pel(*9*)+Cya(*1*)	Pelargonidin-diglycosid (*9*)+Cyanidin-3-monoglycosid (*1*)	ABc	45
Cya+Del(*wenig*)	————————	ABc	46
Cya (*10*)	Chrysanthemin (*10*)	A	47
Cya (*10*)	Cyanidin-3-monohexosid (*10*)	AB	48
Mal (*10*)	Malvidin-glycosid (*10*)	C	49
Mal (*9*)+Del (*1*)	Malvidin-glycosid (*10*)+Delphinidin-glykosid (±)	bC	50
Mal (*10*)	Malvidin-monoglycosid(*5*)+Malvin(*3*)+anderes Glycosid(*2*)	BC	51
Cya (*10*)	Cyanidin-3-monohexosid (*10*)	BC	52
Cya (*10*)	Idaein (*10*)	AB	53
Mal (?)(*10*)	Malvidin-glycosid (?)	A	54
Cya (*10*)	Cyanidin-hexopentosid (*10*)+ 3-monohexosid (±)	D	55
Del (*10*)	Delphinidin-glycosid (*10*)	cD	56
Del (*10*)	Acyliertes Delphinidin-glycosid (*7*)+ 2 Del-glycoside (*2+1*)	Bc	57
Del (*10*)	Delphinidin-glycoside (in 4 Farbstoff-flecken)	bCd	58
Del (*10*)	Delphinidin-glycosid (*7*)+anderes Glycosid (*3*)	Bc	59
Del (*10*)	Delphinidin-dihexosid	B	60
Päo(*9*)+Cya(*1*)	Päonin (*6*)+Päondin-glycosid (*4*)+unbek. Glycosid (±)	D	61
Päo(*9*)+Cya(*1*)	Päonin (*5*)+ Päonidin-glycorid (*5*)+unbek. Glycosid (±)	D	62
Cya (*10*)	Cyanidin-3-hexopentosid (Keracyanin?)(*8*)+Chrysanthemin (*2*)	CD	63
Cya (*10*)	Cyanidin-dihexosid? (*7*)+Cya-monohexosid? (*3*)	CD	64
Del (*10*)	Delphinidin-glycosid (*10*)	AB	65
Del (*10*)	Delphinidin-glycoside (in 2 Formen)	CD	66
Del+Mal (±)	Delphinidin-glycosid (*10*)+Malvidin-glycosid (±)	CD	67
Del(*9*)+Cya(*1*)	Delphinidin-glycosid (*8*)+Cyanidin-glycosid (*2*)+unbekante Glycoside (±)	D	68
Cya (*10*)	Cyanidin-dihexosid (*9*)+Cya-monohexosid (*1*)	bC	69
Cya (*10*)	Cyanidin-hexopentosid (Keracyanin?) (*10*)	AB	70
Cya (*10*)	Cyanidin-3-monohexosid (*8*)+Cya-glycosid (*2*)	BCd	71

Nr.	Familien	Versuchspflanzen	Pflanzenteil	Farbe
72		*Veronicastrum sibiricum* Pennell var. *japonicum* Hara　　クガイソウ	〃	purpur
73	Orobanchaceae	*Aeginetia sinensis* G. Beck.　オオナンバンギセル	Blüten	purpur~ rotviolett
74	Caprifoliaceae	*Sambucus sieboldiana* Blume　　　ニワトコ	Frucht	rot
75		*Viburnum furcatum* Blume　　　オオカメノキ	〃	〃
76	Dipsacaceae	*Scabiosa japonica* Miq.　　　マツムシソウ	Marginal- blüten	hell blau
77	Campanulaceae	*Adenophora nikoensis* Franch. et Sav. 　　　　　　　　ヒメシャジン	Petale	violettblau
78		*A. remotiflora* Miq.　　　　　ソバナ	〃	〃
79		*Campanula dasyantha* Marsch. v. Bieb. 　　　　　　　チシマギキョウ	〃	blau
80		*C. lasiocarpa* Cham.　　　イワギキョウ	〃	〃
81		*C. punctata* Lamarck　　　ホタルブクロ	〃	schwach rotpurpur
82		*Lobelia sessilifolia* Lamb.　　サワギキョウ	〃	violettblau
83		*Phyteuma japonicum* Miq.　　シデシャジン	〃	〃
84		*Platycodon grandiflorum* A. DC.　キキョウ	〃	〃
85	Compositae	*Aster ageratoides* Turcz. var. *ovatus* Nakai 　　　　　　　　　ノコンギク	Blüten	blauviolett
86		*A. tataricus* Linn. fil.　　　　シオン	〃	〃
87		*Cirsium japonicum* DC.　　　ノアザミ	〃	purpurrot
88		*C. nikkoense* Nakai　　　ニッコウアザミ	〃	〃
89		*C. nikkoense* var. *niveum* Kitam.　オキナアザミ	Blüten	purpurrot
90		*Eupatorium glehni* Fr. Schm.　ヨツバヒヨドリ	〃	〃
91		*E. lindleyanum* DC.　　　サワヒヨドリ	〃	〃
92		*Senecio flammeus* Turcz. 　var. *glabrifolius* Cufodontis　コウリンカ	Zungen- blüten	(orange) rot
93	Liliaceae	*Clintonia udensis* Trautv. et Meyer 　　　　　　　ツバメオモト	Frucht	tief blau
94		*Fritillaria camtschatcensis* Ker-Gawl.*　クロユリ	Blumenblatt	dunkel braunrot
95		*Hosta lancifolia* Engler 　var. *thunbergiana* Stearn　コバギボウシ	〃	hell violett
96		*H. longissima* Honda 　var. *brevifolia* F. Maekawa　ミズギボウシ	〃	hell rotviolett
97		*Lilium leichtlinii* Hook. fil. 　var. *tigrinum* Nichols.　コオニユリ	〃	orangerot
98		*L. medeoloides* A. Gray　　クルマユリ	〃	〃
99		*Scilla scilloides* Druce　　　ツルボ	〃	hell purpur
100		*Streptopus streptopoides* Frye et Rigg 　var. *japonicus* Fassett　タケシマラン	Frucht	rot
101		*Trillium smallii* Maxim.　　エンレイソウ	Fruchthaut	schwarzrot
102		*Veratrum japonicum* Loesen. fil.　シュロソウ	Blumenblatt	dunkel rotbraun
103	Iridaceae	*Iris ensata* Thunb. var. *spontanea* Nakai 　　　　　　ノハナショウブ	〃	purpur~ violett
104	Orchidaceae	*Amitostigma keiskei* Schltr.　イワチドリ	〃	rotviolett
105		*Orchis aristata* Fisch.　ハクサンチドリ	〃	〃

* Getrocknete Blüten wurden von Herrn Dr. M. Shibata (Toyama Univ.) uns zur Verfügung gestellt.

Anthocyanidin-Bestandteil	Anthocyaninkomponente	Lokalisation d. Pflanze	Nr.
Cya (*10*)	Cyanidin-glyosid	BC	72
Cya (*10*)	Keracyanin ? (*9*)+Cyanidin-monohexosid (*1*)	D	73
Cya (*10*)	2 Cyanidin-glycoside (*6+4*)	CD	74
Cya (*10*)	Cyanidin-hexopentosid ? (*10*)	BC	75
Del (*10*)	Delphinidin-glycoside (hauptsächl. Delphin ?)	CD	76
Del (*10*)	Delphinidin-glycoside (4 Farbstoff-flecken)	AB	77
Del(*10*)+Cya(±)	2 Delphinidin-glycoside+unbekantes Glycosid (*Spur*)	BCD	78
Del (*10*)	Delphinidin-glycoside (in 2 Formen)	A	79
Del (*10*)	Delphinidin-glycoside (in 3 Formen) (*8+1+1*)	A	80
Cya(*9*)+Del(*1*)	Keracyanin? (*7*)+Delphinidin-glycosid (*3*)	bCD	81
Del(*10*)+Cya(±)	Delphinidin-glycoside (in 2 Formen) (*7+3*)	C	82
Del (*10*)	Delphinidin-glycosid (*10*)	D	83
Mal(*7*)+Del(*3*)	Malvidin-glycosid (*6*)+Delphinidin-glycosid (*4*)	cD	84
Cya(*9*)+Del (*1*)	Cyanidin-dihexosid (*8*)+Delphinidin-dihexosid (*2*)	CD	85
Del (*10*)	Delphindin-dihexosid ? (*8*)+Delphinidin-glycosid (*2*)	D	86
Cya (*10*)	Cyanidin-dihexosid (*9*)+Chrysanthemin (*1*)	CD	87
Cya· (*10*)	Cyanidin-dihexosid(Komplex ?)(*10*)+2 unbek. Glycoside(*Spur*)	BC	88
Cya (*10*)	Cyanidin-3·5-dihexosid (*8*)+Chrysanthemin (*2*)	BC	89
Cya (*10*)	Chrysanthemin (*10*)	BCD	90
Cya (*10*)	" (*10*)	D	91
Cya (*10*)	" (*7*)+Cyanidin-glycosid (*3*)	BC	92
Mal(*10*)+Del(±)	Malvidin-3-monohexosid (*10*)+unbek. Glycosid (±)	B	93
Cya (*10*)	Keracyanin ? (*8*)+Chrysanthemin (*2*)	A	94
Mal (*10*)	Malvidin·3·5-dihexosid (*8*)+Mal-glycosid (*2*)	C	95
Mal (*10*)	Malvidin-3·5-dihexosid + 2 andere Mal-glycoside	CD	96
Cya (*10*)	Keracyanin? (*10*)+ andere Cyanidin-glycoside (*Spur*)	bCd	97
Cya (*10*)	" (*10*)+Cyanidin-3-monohexosid? (±)	BC	98
Cya (*10*)	Cyanidin-glycoside (in 2 Farbstoff-flecken)	D	99
Cya (*10*)	Cyanidin-glycosid (Chrysanthemin ?)	BC	100
Cya (*10*)	Cyanidin-3-hexopentosid (*10*)+3-monohexosid (±)	bCD	101
Cya (*10*)	Cyanidin-glycoside (in 4 Farbstoff-flecken)	BCd	102
Mal (*10*)	Malvidin-glycosid (Ensatin ?)	Cd	103
Cya (*10*)	Cyanidin-dihexosid (*10*)+unbekanntes Glycosid (*Spur*)	C	104
Cya (*10*)	" (*10*)+2 Cya-glycoside (±)	Bc	105

TdicSa F₁ に *Haynaldia* をかけて得られた 1 植物

中　島　吾　一*

Goichi NAKAJIMA: The Parthenogenesis of Wheat-rye Hybrid by the
Influence of the Pollen of *Haynaldia villosa*.

緒　　言

1953〜54 年に得た *Triticum dicoccum* var.
atratum × *Secale africanum* F₁ (TdicSaF₁) は
高い稔性（小穂に対する稔率 37.94〜79.27 平均
58.11%）を示し，F₂ で amphidiploid を生じた
(Nakajima 未発表)。1954 年に 3 属間雑種を作
出する目的でこの F₁ の穂 20 本を用い，その
700 花を *Haynaldia villosa* の花粉で授粉した。
この交配では 3 属間雑種は得られなかつたが，
2n＝20 の染色体を持つ 1 個体が得られた。ここ
にこの個体について行つた細胞遺伝学的研究の結
果を述べる。

観　察　結　果

A.　2n＝20 植物の外部形質:

2n＝20 植物の外部諸形質を TdicSaF₁, F₂ お
よびその両親の外部諸形質と比較すれば第 1 表の
通りである。

　第 1 表に示すように，この 2n＝20 植物の外
部諸形質は TdicSa F₁, F₂ および両親のそれに
比較して 1〜2 の形質を除いてはすべてそれらよ
りも劣つていた。概括的に見てこの植物はやや小
型であるだけで F₁ および F₂ に似ていた (Fig.
1)。穂の色は F₁ および F₂ とほとんど同じであ
る。

　B.　2n＝20 植物の PMC における成熟分裂:
　この植物の根端細胞で，20 の染色体が明かに
観察された (Fig. 2)。その PMC においてもま
た 2n 数として 20 の染色体が観察された (Figs.
3〜6)。また第 1 成熟分裂中期で，0〜3 の 2 価

Table 1.　External characters of 2n＝20 plant compared to the TdicSa F₁, F₂
and its parental plants.

Characters / Plants	Number of chromosomes 2n	Length of culms cm	Length of spikes cm	Length of awns cm	Number of spikelets per spike	Spike density	Number of kernels per spike	Percentage of seed setting per spikelet	Number of flowers per spikelet	Number of tillerings
T. dicoccum var. *atratum*	28	131.46	10.37	10.78	29.05	2.80	44.60	153.18	3	45.44
S. africanum	14	130.40	13.60	0.00	50.40	3.71	30.19	59.95	2	100
F₁	21	152.00	14.10	4.11	40.86	2.90	23.80	58.11	4	83.00
F₂	42	135.12	15.97	4.87	34.84	2.18	37.66	108.09	3	58.30
2n＝20 plant	20	106.50	11.20	3.10	30.40	2.67	0.40	1.32	3	71.00
Haynaldia villosa	14	114.30	7.89	3.73	22.25	2.82	26.67	119.44	5	

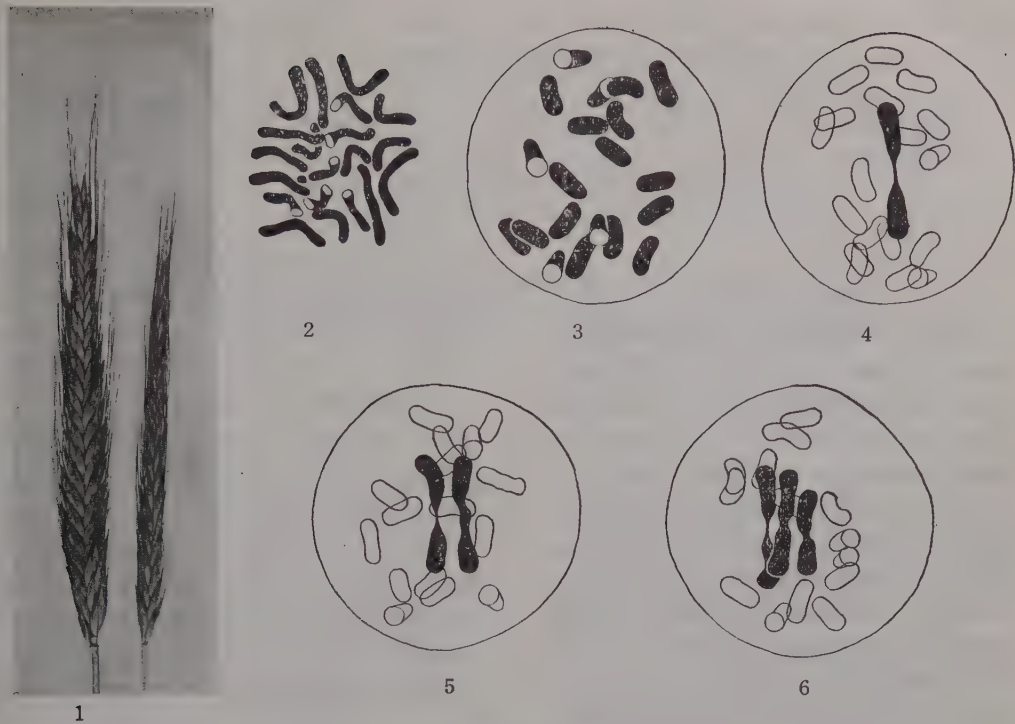* 群馬大学工学部生物学教室. Biological Laboratory, Faculty of Technology, Gumma University.

Fig. 1.   Spikes of a plant having 2n=20 chromosomes and its parent (TdicSaF₁), left
         TdicSa F₁, right 2n=20 plant, ×ca 1/2.

Fig. 2.   Somatic plate in root tip cells of 2n=20 plant. ×1000.

Figs. 3~6.   Meiosis in PMC's of 2n=20 plant. ×770.

        3.   Side view of heterotypic metaphase, 20 univalents scattered in spindle.

        4.   do. $1_{II}+18_I$.

        5.   do. $2_{II}+16_I$.

        6.   do. $3_{II}+14_I$.

Table 2.   Frequency of bivalents at heterotypic metaphase in meiosis of PMC's
of 2n=20 and its mother plant.

Bivalents Plants	0_{II}	1_{II}	2_{II}	3_{II}	Mode (%)	Total (%)
2n=20 plant	698 (69.80)	271 (27.10)	30 (3.00)	1 (0.10)	0_{II} (69.80)	1000 (100.00)
TdicSaF₁ (2n=21)	2853 (51.87)	2192 (39.86)	416 (7.56)	39 (0.71)	0_{II} (51.87)	5500 (100.00)

染色体と 14~20 の 1 価染色体とを観察した
(Figs. 3~6)。1 母細胞中に見出される 2 価染色
体数の頻度は第 2 表の通りである。

　第 1 分裂後期における染色体の両極への分配は
機会的に行われ，3：17~10：10 迄の場合が見ら
れ，その頻度は第 3 表の通りである。

　上述のように，この 2n=20 染色体を持つ個体
における成熟分裂は，多くの小麦ライ属間雑種 F₁
におけると同様で，赤道板の形成等も少なく，生
ずる花粉はほとんど受精力を欠き高い不稔性を示
し，僅かに 1~2 粒の種子を得たにすぎない。

Table 3. Distribution of the chromosomes to the poles at the heterotypic ana-telophase in PMC's of 2n=20 plant

Distribution of chromosomes	3: 17	4: 16	6: 14	7: 13	8: 12	9: 11	10: 10	Mode	Total
2n=20 plant	3	1	5	5	7	11	23	10: 10	55
%	5.45	1.82	9.09	9.09	12.73	20.00	41.82	41.82	100.00

考 察

　この 2n＝20 染色体を持つ個体は TdicSaF₁ に Haynaldia villosa の花粉を授粉して得たものであつて，真正三属間雑種であるならばその染色体数は2n＝28 でなければならない。この植物の外部形態は小型ではあるが TdicSaF₁ に酷似しているが，T. dicoccum var. atratum × H. villosa F₁ (Nakajima 未発表) とはかなり異つており，Haynaldia の形質は包含されていない。

　このように，外部諸形質，染色体数および花粉母細胞成熟分裂の観察結果等から考察して，この 2n＝20 染色体の植物は TdicSaF₁ に H. villosa の花粉を授粉して生じたものであるが，Triticum-Secale-Haynaldia 三属間雑種ではなく，TdicSaF₁ の胚嚢母細胞が花粉母細胞の成熟分裂と同様の過程を経て生じた 20 の染色体を持つ卵が，Haynaldia の花粉を授粉することによつてその刺激を受け単為生殖によつて発生したものであつて，そのゲノム構成は ABRᵃ ―1＝20 であると考えられる。すなわちこの個体は TdicSa 複二倍体植物の hypo haploid に相同のものといい得よう。

　このような一倍体はすでに Aegilotricum (Katayama 1935), Timopheevi-turanicum amphidiploid (Sachs 1952) および Timopheevi-squarrosa amphidiploid (Watanabe and Mukade 1955) においても報ぜられている。

Résumé

1. In the present report, a result of cytogenetical study on a plant derived from TdicSa F₁ (*Triticum dicoccum* var. *atratum* × *Secale africanum* F₁) after the pollination with *Haynaldia villosa* is described.

2. The external characters of this plant, resemble these of TdicSa F₁ in every point though somewhat inferior as shown in Table 1 and Fig. 1.

3. The number of somatic chromosomes of the plant is distinctly found to be 20 (Fig. 2).

4. The number of bivalents in one PMC at heterotypic metaphase varies from 0 to 3, and the frequency of it is tabulated in Table 2, 0_{II} being the mode. The results are the same as in the parent TdicSa F₁.

5. Considering from the external characters and the meiosis of PMC's, this plant (having 2n＝20 chromosomes) may be considered as having developed by parthenegenesis, influenced by the pollen of *Haynaldia villosa*, from an egg having 20 chromosomes.

Literature Cited

1) Katayama, Y., Jap. Jour. Bot. 7: 349 (1935).　　2) Sachs, L., Heredity 6: 157 (1952).　　3) Watanabe, Y. and Mukade, K., Jap. Jour. Breed. 5: 75 (1955).

キンボウゲ科の細胞学的研究 X

イチリンソウ属，サラシナショウマ属およびセンニンソウ属の核型続報

栗 田 正 秀*

Masahide KURITA: Cytological Studies in Ranunculaceae X.
Further Notes on the Karyotype of *Anemone, Cimicifuga* and *Clematis*.

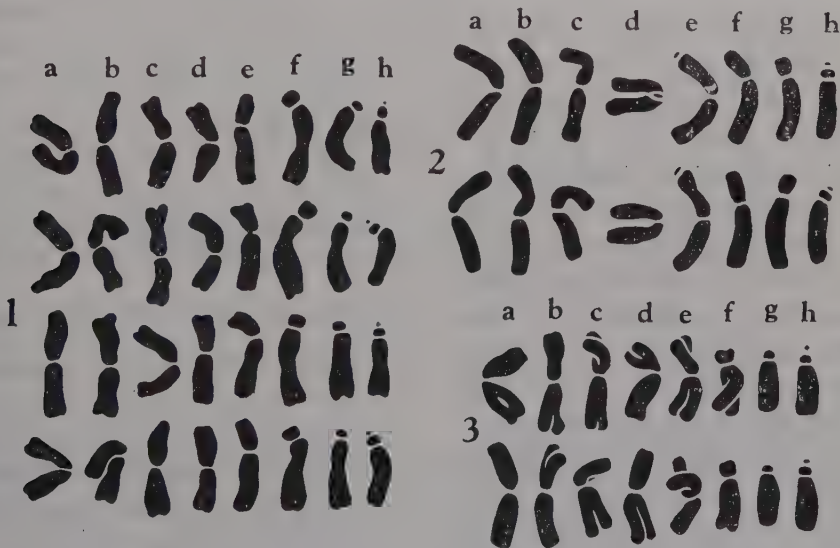筆者 (1955 a, c, d, 1956) はさきにイチリンソウ属 (*Anemone*)，サラシナショウマ属 (*Cimicifuga*) およびセンニンソウ属 (*Clematis*) の核型について報告した。その後，さらにこれら 3 属に属する数種の核型を分析することができたのでその結果と各属の基本染色体組の比較についてのべる。

方法は前報告におけると同じである。

観　察

1. キクザキイチゲ　*Anemone pseudo-altaica* Hara (北海道音江村産)，アズマイチゲ *A. Raddeana* Reg. var. *integra* Huth. (三重県藤原岳産)

キクザキイチゲの根端細胞染色体は 32 個で，これらは構成員のそれぞれ一致する 4 組にわけ

Figs. 1-3. Somatic chromosomes   1, *Anemone pseudo-altaica*   2, *Cimicifuga acerina*
var. *peltata* 3, *Clematis alpina* var. *ochotensis*   ×1720.

* 愛媛大学文理学部生物学教室
  Biological Institute, Faculty of Science and
  Literature, Ehime University, Matsuyama,
  Japan

ることができ，各組の 8 染色体はさらに次のように 4 群に大別できる。1) 中部着糸の 4 染色体 (第 1 図，a–d)，これらは順次長さにわずかの差がある。2) 次中部着糸の 1 染色体 (同，e)，

長さは前群の最小染色体ぐらいで，短腕は長腕の 1/2 よりわずかに長い。3) 次端部着糸の 1 染色体 (同，f)，この短腕は染色体の幅ぐらいの長さがある。4) 次端部着糸の 2 染色体 (同，g および h)，いずれもその短腕はあきらかに第 3 群染色体のそれより短く，うち 1 個ではその短腕に小さい付随体がみとめられる。以上のべたキクザキイチゲの基本染色体組はさきに筆者 (1955 a) が報告したサンリンソウ (A. stolonifera) の半数染色体組によく似ている。

キクザキイチゲとアズマイチゲとの間に核型の相違はみとめられなかつた。したがつて両植物の核型は次のようにしめせる。

$$K(2n) = 32 = 16A^m + 4B^{sm} + 4C^{st} + 4D_1^{st} + 4^tD_2^{st}$$

2. オオバショウマ Cimicifuga acerina (Sieb. et Zucc.) Tanaka var. macrophylla (Koidz.) Hara (栃木県日光産)，キケンショウマ C. acerina (Sieb. et Zucc.) Tanaka var. peltata (Makino) Hara (長野県駒岳産)

杉浦 (1937, 1939) はオオバショウマの染色体数を n=8 と報告した。

キケンショウマの体細胞染色体は 16 個で，これらはその形態から次のように 4 群に大別できる。1) 中部に着糸点をもつ 5 対の染色体 (第 2 図，a-e)，各対の大きさは大差なく，うち 1 対ではその 1 腕末端に付随体が存在する。2) 次中部着糸の 1 対 (同，f)，短腕の長さは長腕のほぼ 1/2 で，5 核板における測定の平均ではわずかに長い結果をえた。3) 次端部着糸の 1 対 (同，g)，短腕は染色体の幅よりやや長い。5) 次端部着糸の 1 対 (同，h)，この短腕は染色体の幅よりあきらかに短く，かつ付随体をもつので前者との区別は容易である。

キケンショウマとオオバショウマとの間で核型上の差異はみとめえなかつた。したがつて両変種の核型は次のようにしめせる。

$$K(2n) = 16 = 8A_1^m + 2^tA_2^m + 2B^{sm} + 2C^{st} + 2^tD^{st}$$

上述の核型はさきに筆者 (1955 d) が報告したキレハオオバショウマ (C. acerina var. intermedia) のそれに似ている。

3. イヌショウマ C. japonica (Thunb.) Spreng. (奈良県西原村産)

本種の核型はキケンショウマのそれに非常によく似ているが，中部着糸の染色体で付随体がみとめられなかつた点で両者はことなる。

したがつてイヌショウマの核型は次の式でしめせる。

$$K(2n) = 16 = 10A^m + 2B^{sm} + 2C^{st} + 2^tD^{st}$$

4. ミヤマハンショウヅル Clematis alpina (L.) Mill. var. ochotensis (Pall.) O. Kuntze (栃木県日光産)，タカネハンショウヅル C. lasiandra Maxm. (徳島県祖谷渓産)，キイセンニンソウ C. ovatifolia Ito (和歌山県那智山産)

ミヤマハンショウヅルの根端細胞で筆者は酒井 (1935) と同じく，16 個の染色体をみとめた。これらはその形態から次のように 3 群に大別することができる。1) 中部着糸の染色体 5 対 (第 3 図，a-e)，各対は順次長さにわずかの差がみられる。2) 次端部着糸の 1 対 (同図，f)，短腕の長さは染色体の幅ぐらいある。3) 次端部着糸の 2 対 (同図，g および h)，いずれの短腕もその長さは染色体の幅よりあきらかに短く，1 対では付随体がみとめられる。

タカネハンショウヅルとキイセンニンソウの核型はともに上述のミヤマハンショウヅルのそれによく似ていて差異はみとめられなかつた。したがつてこれら 3 者の核型は次のようにしめせる。

$$K(2n) = 16 = 10A^m + 2B^{st} + 2C_1^{st} + 2^tC_2^{st}$$

上述の核型は筆者 (1955 c) がさきに報告したテッセン (C. florida) のそれによく似ている。

5. C. ligusticifolius Nutt. (米国産)

本種の核型はミヤマハンショウヅルのそれに似ているが次の点，すなわちミヤマハンショウヅルの第 3 群染色体に相当する C. ligusticifolius の染色体では 2 対ともにその短腕に付随体が存在する点で両者はことなつている。

したがつて核型は次の式であらわせる。

$$K(2n) = 16 = 10A^m + 2B^{st} + 4^tC^{st}$$

Meurman および Therman (1939) は本種で

1 対の付随体染色体しかみとめていない。

6. クサボタン C. stans Sieb. et Zucc.（新潟県杉之沢村産）

本種の核型もミヤマハンショウヅルのそれによく似ているが次の点だけでことなる。すなわちミヤマハンショウヅルの第 3 群染色体で付随体のない 1 対に相当するクサボタンの 2 染色体のうち，一方にはその短腕に大きい付随体がある点である。したがつてクサボタンには 3 個の付随体染色体が存在するが，そのうちの 2 個はミヤマハンショウヅルのそれによく似ていて，付随体は小さく，残り 1 個はミヤマハンショウヅルではみとめられない大きい付随体をもつている。

したがつて本種の核型は次の式でしめせる。

$$K(2n) = 16 = 10A^m + 2B^{st} + C_1^{st} + {}^tC_2^{st} + 2{}^tC_3^{st}$$

中島 (1937) は本種の雄株で 2n=16, n=8 の染色体数を決定し，減数第 1 分裂で不等対はみとめなかつた。筆者の材料はいまだ開花しないので

比較を簡明にするために中部および次中部着糸の染色体をそれぞれ単に m および sm で，次端部着糸で短腕の長さが染色体の幅ぐらいかまたはそれよりやや長い染色体を st_1，同じく幅よりあきらかに短い染色体を st_2 でしめすとサラシナショウマ属の基本染色体組は $5m + sm + st_1 + st_2$，センニンソウ属のそれは $5m + st_1 + 2st_2$，イチリンソウ属のそれは $4m + sm + st_1 + 2st_2$ である（第4 図）。上記で明らかなように $5m + sm$ をもつのはサラシナショウマ属だけであるが，その sm は既述のように長腕の 1/2 よりわずかに長い短腕をもち st_1 に近い形態であつて，この sm にかわつて st_2 がはいつたものがセンニンソウ属の基本染色体組となる。したがつて両染色体組の相違はわずかであつて両属間の近縁な関係を示すものと考えられる。分類学上センニンソウ属に近い位置におかれている (Prantl 1891) イチリンソウ属では $5m$ のかわりに $4m + sm$ となつているだけでわずかにセンニンソウ属とは異つている。しかしイチリンソウ属一部の種ではその sm が st_1 に類

Fig. 4. Diagrams representing basic complements   a, *Cimicifuga,*   b, *Clematis*   c, *Anemone* (with b=8).

雌雄の別は不明であり，上述の大きい付随体について不等である 1 対の染色体が性染色体であるかどうかはなお今後の研究にまちたい。

考　察

本報告の種をくわえて筆者はこれまでにイチリンソウ属（b=7 の系統は除く）7 種 3 変種，サラシナショウマ属 1 種 4 変種，センニンソウ属 8 種 2 変種 1 品種の核型を分析した。付随体を考慮せず，これら 3 属の基本染色体組を比較してみよう。

似する形態となり，$4m + 2st_1 + 2st_2$ に近いものがある。上述からあきらかなようにこれら 3 属の基本染色体組は似てはいるが，サラシナショウマ属からセンニンソウ属をへてイチリンソウ属にいたるにしたがい或る染色体における非相称の程度がわずかに高くなつているのがみとめられる。

なおイチリンソウ属内一部の種の基本染色体組が $4m + 2st_1 + 2st_2$ に近い形態をしめすことは，それらがほぼ同じ式でしめせる基本染色体組をもつキンポウゲ属 (*Ranunculus*) の一部（未発表）あるいはモミジカラマツ属 (*Trautvetteria*) に対

し類縁関係をしめすものではなかろうか。オキナ
グサ属 (*Pulsatilla*) (筆者 1956) の基本染色体組
はイチリンソウ属のそれとよく似ている。センニ
ンソウ属とレンゲショウマ属 (*Anemonopsis*) と
の関係, イチリンソウ属 b=8 の系統と b=7 の

系統またはユキワリソウ属 (*Hepatica*) との関係
についてはさきにのべた (筆者 1955 a, b)。

Summary

1. Karyotype analysis was carried out on nine species and one variety. The results obtained are as follows:

Anemone pseudo-altaica
A. Raddeana var. *integra* $\Big\}$ $K(2n)=32=16A^m+4B^{sm}+4C^{st}+4D_1^{st}+4^t D_2^{st}$

Cimicifuga acerina var. *macrophylla*
C. acerina var. *peltata* $\Big\}$ $K(2n)=16=8A_1^m+2^tA_2^m+2B^{sm}+2C^{st}+2^tD^{st}$

C. japonica 　 $K(2n)=16=10A^m+2B^{sm}+2C^{st}+2^tD^{st}$

Clematis alpina var. *ochotensis*
C. lasiandra
C. ovatifolia $\Bigg\}$ $K(2n)=16=10A^m+2B^{st}+2C_1^{st}+2^tC_2^{st}$

C. ligusticifolius 　 $K(2n)=16=10A^m+2B^{st}+4^tC^{st}$

C. stans 　 $K(2n)=16=10A^m+2B^{st}+C_1^{st}+{}^tC_2^{st}+2^tC_3^{st}$

2. When a satellite is left out of consideration, the basic chromosome set of *Cimicifuga* differs from that of *Clematis* in having one chromosome with a submedian constriction instead of one chromosome with a short arm remarkably smaller than chromosome–breadth, and the difference in the basic set between *Clematis* and *Anemone* (with b=8) lies in the fact that the former has one chromosome with a median constriction in place of one chromosome with a submedian constriction in the latter (cf. Fig. 4).

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ヤマノイモとシュウカイドウのシュウ酸カルシウムの結晶

高　見　　亘*

Wataru TAKAMI: Calcium Oxalate Crystals in *Dioscorea japonica* Thunb. and *Begonia Evansiana* Andr.

無機塩の欠乏による影響については，すでに多くの研究者によって研究されているが，シュウ酸カルシウムの結晶形成への影響については殆どなされていない。筆者[5,6]はこれまでに簡単な観察を行ったことがあるが，ここには結晶が豊富でその分布の観察に便利なヤマノイモと前報[6]のイオンによる晶癖をさらに確かめるのに便利なシュウカイドウについて調べた結果を報告する。

材 料 及 び 方 法

子葉だけが見られたヤマノイモの芽生え 2 本ずつを 5 月下旬に，次の 7 種の培養液を毎日更新した 500 c.c. のフラスコで水耕した。

(1) Control としてクノープ液 (pH 5.0 の井戸水を使う)，(2) K 欠如液 ($Ca(NO_3)_2$ 1 g, $MgSO_4$ 0.25 g)，(3) S 欠如液 ($CaCl_2$ 1g, KCl 0.25 g)，(4) N 欠如液 (KH_2PO_4 0.25 g, $CaCl_2$ 1g)，(5) Mg 欠如液 ($CaSO_4$ 1 g, KCl 0.25 g) (6) K, S 欠如液 ($CaCl_2$ 1 g, $MgCl_2$ 0.25 g)，(7) K, S, Mg 欠如液 ($CaCl_2$ 1 g, $(NH_4)_2HPO_4$ 0.25 g)

シュウカイドウの場合には次の 5 種を用いた。(1) クノープ液，(2) K 欠如液，(3) N 欠如液，(4) Mg 欠如液，(5) 少量のアンモニア水を加えたクノープ液。

前者は約 3 週間後，後者は 4 週間後に抱水クロラールで透明にして検鏡し，さらに，自生及び栽培されたヤマノイモについても調べた。

* 早稲田大学商学部生物学教室
Biological Institute, Faculty of Commerce, Waseda University, Tokyo

実 験 結 果

(1) 発育に対する無機塩の影響

ヤマノイモとシュウカイドウではかなりの差異があり，前者では K 欠如液でも発育は大いによく，Mg 欠如液も著しい影響を与えないが，S または N 欠如液では殆ど生長せず，子葉以外に 1〜2 葉が出ただけで蔓は伸長しなかった。根の伸長も同様な影響が見られたが，K 欠如液ではとくに伸長した。後者では，K 欠如液では発育甚だ不良で，根は萎縮してとれてしまい，新しく補充しても同様であったが，N 欠如液ではかえって発育良く，Mg 欠如液では著しい変化はなく，アンモニア水混入のものは発育良好であった。根の伸長は N 欠如液とアンモニア水混入のものが良好であった。

(2) ヤマノイモにおける結晶の分布

結晶形はすべて針状で根茎には多くなく，維管束の周辺に見られた。稚葉 (0.5 cm 以下) では最初に葉脈の周辺と葉縁に現われ，中間の葉肉部には見られなかった。葉序による分布の変化は，地上茎が 20 cm のものでは，頂上の甚だ小さな 2, 3 葉を除いて数えた第 3 葉 (0.5 cm) では中間の葉肉部になく，第 4 葉 (1 cm) では直径 650μ の視野 (以下同様とする) に 23〜35μ の大形のとそれより小形の針状束が 18〜19 個位見られ，第 5 葉 (1.5 cm) では大形のは 58〜69μ になって 10 回の測定での度数分布は 11 (1 回)，14 (2 回)，15 (5 回)，16 (1 回)，19 (1 回) であった。次の第 6, 7, 10 葉について葉長，大形の結晶の長さ，度数分布は表 1 のようで，針状束

はしだいに太く同様なものになった。地上茎 30 cm のものでは、中間の葉肉部の結晶は第 5 葉 (0.6 cm) に至って現われ、第 7 葉 (0.9 cm) では 24～30 個位で甚だ多く、第 8 葉 (1.4 cm) で分布を調べると 11 (2 回)、12 (1 回)、13 (1 回)、16 (1 回)、17 (2 回)、18 (1 回)、19 (1 回)、20 (1 回) であった。次の第 10, 14, 24 葉の測定は表 1 のようであった。

このように、始めは中間の葉肉部に結晶はなく、数番目の葉で密度は最大となり、その後減少し、この第 1 例で、第 5, 第 6 葉の表皮細胞の伸長の比は約 3：4 であった。栽培されたものでは、密度は小で、第 4 葉 (1 cm) のものでは 35～46μ の針状束が 2～3 個、第 5 葉 (1.5 cm) では 6 個位であった。次の第 5, 9, 11 葉に対する結果を表 2 に示す。

表 1. ヤマノイモのシュウ酸カルシウム結晶の分布　（直径 650μ の視野）

	茎　長　20 cm			茎　長　30 cm		
葉　　　序	6	7	10	10	14	24
葉　長 (cm)	3.0	4.0	5.5	3.6	4.5	5.5
結晶の長さ (μ)	81～92	116～127	150～173	116～127	116～127	116～127
1 個			1			
2			11			1
3		2	8		4	10
4		2		1	10	6
5	2	5		5	5	2
6	1	7		5	1	1
7	5	2		6		
8	3	2		3		
9	7					
10	2					
平　　　均	7.9	5.5	2.4	6.3	4.2	3.6

第 2. 栽培されたヤマノイモの結晶の分布

葉　　　序	5	9	11
葉　長 (cm)	1.5	1.5	2.5
結晶の長さ (μ)	35～46	58～66	58～66
1 個			2
2			9
3			3
4	2	1	6
5	8	4	
6	4	4	
7	2	7	
8	4	3	
9		1	
平　　　均	5.9	6.5	2.7

(3)　環境による結晶分布の変異

水耕の場合に同時測定をしたので葉長は等しくないが、測定結果は表 3 及び Figs. 1～2 のようであった。K, S, Mg 欠如液で、葉長 1.3 cm のものの結晶長は 35～46μ で密度大の為に表から除いたが、結果は 13 (1 回)、16 (1 回)、18 (1 回)、19 (1 回)、20 (1 回)、21 (3 回)、22 (2 回)、24 (2 回)、25 (1 回)、27 (3 回)、28 (1 回)、30 (1 回)、31 (1 回)、33 (1 回) であった。この比較に用いた自生のものは他の水耕のもの及び前に調べた自生のものより密度が大で土地の状態による変異はかなり大きいと考えられる。
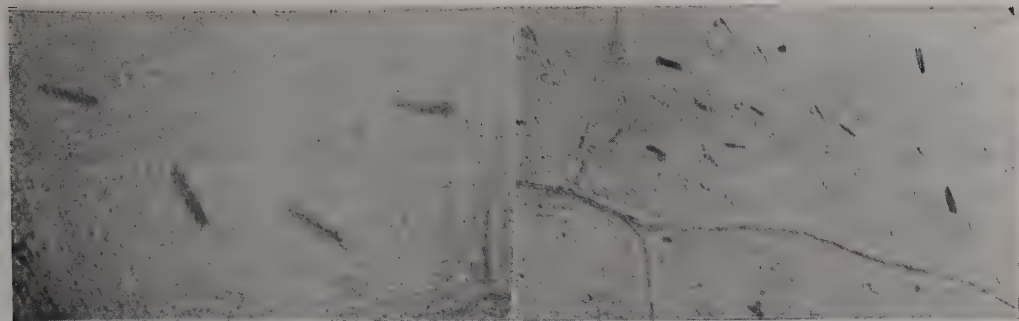
次に、アスファルトの道に生えて発育不良のもの、K 欠如液に少量のショ糖

Fig. 1.　　　　　　　　　　　　　　　Fig. 2.

Figs. 1-2. Distribution of calcium oxalate crystals in *Dioscorea japonica* Thunb.
Fig. 1. In the case of absence of sulphur (leaf length 1.3 cm).
Fig. 2. In the case of absence of potassium, sulphur and magnesium
(leaf length 1.3 cm). ×100.

表 3. 環境の差によるヤマノイモの結晶の分布の変異

	自　生	クノープ液	K 欠	S 欠	N 欠	Mg 欠	K, S 欠
葉　　長 (cm)	2.5	2.5	2.5	1.3	3.8	2.5	1.0
結晶の長さ (μ)	104〜116	104〜116	104〜116	81〜92	127〜139	81〜107	92〜104
1 個		2					
2		4			4		
3		8			12		
4		5		3	4	2	
5		2		12		6	
6			4	5		5	
7			7			4	
8	1		2			3	2
9	2		4				1
10	2		2				1
11	4						3
12	6		1				2
13	3						
14	2						
平　　均	11.5	3.2	7.9	6.1	3.0	6.0	9.2

をいれて根が発育不良のものでは小形の針状束が多数見られ，暗所でクノープ液に培養したものでは稚葉における分布はむしろより多く，ことに葉の先端で著しかった。

（4）シュウカイドウにおける結晶の分布

結晶は 3 分子の結晶水を含む八面体または金米糖状で，茎と葉柄では維管束の周辺に多数見られ，その附近に厚膜細胞が随処にできている。3 葉をつけたもので，0.4 cm の葉では葉脈にそっ

てのみ八面体または結晶砂が見られ，葉の先端と毛の部分にはとくに多かった。次の 2, 7 cm のものになるにつれて結晶は大形となり，ずっと大きい植物では小さい葉でも結晶砂は見られないものもあった。

（5）環境による結晶分布の変異と晶癖

K 欠如液では，葉柄，葉面とも結晶は少なく，N 欠如液では反対に甚だ多く自生のものと似ていた。Mg 欠如液では結晶は小さく，分布も少ない

ようで，ことに葉面では結晶砂程度であった。ア
ンモニア水混入のクノープ液では結晶は大きく，
分布も多かった。

　次に，晶癖に関しては，K 欠如液では柱状のも
のが少数と砂晶が集まったものが 1 個見られ，N
欠如及び Mg 欠如液では小形なだけでふつうの

でも葉肉の部分でシュク酸が生成するのはある程
度細胞分化が行われてからで，結晶密度はその時
期に最大となると考えられる。上の実験結果によ
って，ヤマノイモでは栄養状態が極端に悪ければ
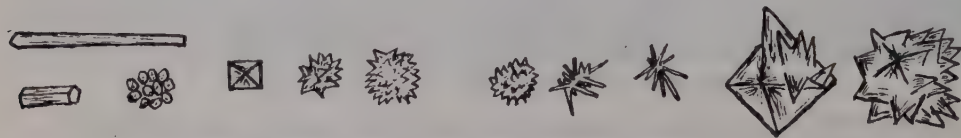結晶生成は減少するが，ある程度の不良では反対
に小形のものが多く作られる。K と Mg とでは

Fig. 3.　　　　　　Fig. 4.　　　　　　　　Fig. 5.　　　　　　Fig. 6.

Figs. 3–6.　Crystal habits of calcium oxalate in *Begonia Evansiana* Andr.　× 480.
Fig. 3.　In the case of absence of potassium.
Fig. 4.　In the case of absence of nitrogen or magnesium.
Fig. 5.　In the case of Knop's solution, containing ammonia water.
Fig. 6.　In the case of an ordinary soil for comparison.

と変りがなく，アンモニア水混入のクノープ液で
は葉面のは変りがなかったが，葉柄では針状束の
ものも見られた (Figs. 3～6)。

考　察　及　び　結　論

　上の観察によって，ヤマノイモとシュウカイド
ウでも他の多くの植物の場合と同様にシュウ酸カ
ルシウムの結晶は最初維管束にそって現われ，葉
肉にはあるとしても甚だ小さな点として存在する
にちがいない。Scott[4] によれば，シュウ酸カル
シウムの結晶を含む異形細胞は脂肪を含むものよ
り遅く発育するが，ヤマノイモとシュウカイドウ

Mg 欠如の方が良好の状態ということができ，K,
Mg, S 欠如では結晶は生長せず，細く短いものが
随処にできて，シュウ酸生成が異常であると認め
られる。また白化葉ではふつうは結晶は少いとさ
れているが[4]，ヤマノイモではむしろ多くなり，
シュウ酸生成が促進されたことを示している。

　ヤマノイモでもシュウカイドウでも表皮系はよ
く発達し，ことに前者の稚葉は柔毛が密生し，葉
縁の表皮細胞はその後によく発達する。後者では
葉の生長につれて剛毛がよく発育して結晶を多数
含む。

Summary

　In the present investigation, the distribution and habit of calcium oxalate crystals in *Dioscorea japonica* Thunb. and *Begonia Evansiana* Andr. were observed.

　(1)　Distribution of the crystals in *Dioscorea* which was grown in the field or grew wild on an ordinary soil was examined.　They appear at first near the nerve and in the epidermis and then in the mesophyll.　Their number in the circle of 650μ diameter increases and decreases after attaining its maximum.　In case of ample supply of nutriment, the number is smaller.

　(2)　Effects of absence of particular elements, K, S, N, Mg are observed, in case of *Dioscorea*, effect of absence of K is not remarkable, while in case of *Begonia* it is very remarkable.　In those cases, distribution of crystals was compared.　Also,

Dioscorea which grew wild under very bad condition was examined. It may be able to say that deficiency of nutriment increases the production of crystals.

(3) In case of *Begonia* the variation of nutriment may be related to the crystal habit and as indicated in the previous paper, action of NH_4^+ in forming raphide was observed.

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本 会 記 事

支部所在地変更

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関東支部

　昭和30年度支部大会（4月22日，於国立科学博物館）石川茂雄，大房剛*：オシダの胞子の発芽におよぼす光の影響．川崎次男：ヘラシダ属数種の有性世代．百瀬静男：配偶体によるシノブ群の分類批判．加埼英男：ホシツリモについて．小山鉄夫：カヤツリグサ科スゲガヤ類の小穂構造について（予報）．壜順：ゴマの生長点の構造と葉形成

の週期について．保井コノ：トウモロコシの花序に現われた変異から推定される雌花穂の発生機構について．秋野善一：ノリの赤グサレ病原菌の生殖について．野沢冷治，野沢ユリ子：アサクサノリ細胞の変形について．三輪知雄，中村佐兵衛，山本茂，山本昭子：作物の登熱過程における窒素代謝（1）稲のアミノ酸合成に関する酵素．高橋基生：珪酸塩のイネ科植物に対する効果について．西岡泰三：タンポポ類の染色体 II.

九州支部

　第39回例会（4月14日，於福岡学芸大）窪田日出夫：蘚生蘚類の滲透価及び耐乾性．清水正元：肥料の種類及び土壌の熟度と雑草の群落．特にメヒシバの分布について．山下孝介：一粒系コムギ相互転座型の核型分析．

投 稿 の 注 意

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（例） Bot. Mag. Tokyo 69: 192 (1956)

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The Taxonomical Observations on the Natural Variation in *Lilium speciosum* Thunb. I*

by Sadao ABE and Teruo TAMURA**

阿部定夫・田村輝夫: カノコユリの自然変異と分類学的考察 I

The studies have been made to solve fundamental problems on the breeding of *Lilium speciosum* since 1948. The details of the studies were written in Japanese, with color plates, under the title " Studies in the natural variation of *Lilium speciosum* " in the " Studies on the Export Bulbs " edited by Dr. M. Sisa, published by Seibundo Co., Tokyo, in March 1955. We thank heartily to Dr. F. Maekawa of the Tokyo University and Mr. S. Kumazawa, chief of Horticultural Division of the Station, for their directions.

Distribution

In China, this lily is found in the Lushan mountains of Kiangsi Province (Wilson 1925, Watson 1931, Wong 1934) and in Formosa, in the vicinity of Taihoku (Hayata 1911, 1912, Wilson 1925). In Japan it is confined to Kyûshû and Shikoku Provinces, and was long obscure. Kaempfer (1712) referred it to Korea, and Thunberg (1784, 1811) mentioned it from Nagasaki, although in the latter case it is not clear whether the plant was wild or cultivated. The first clear reference to the habitat in Japan was made by Makino (1907) who recorded from Kôchi and Nagasaki. Afterwards, many wild habitats have been reported, and there was also found the lily grown wild in Kagoshima Pref. (Wilson 1925, Doi 1925, Naito and Kajiwara 1934) and Tokushima Pref. (Abe 1937). Further, we have newly found the reliable habitats in various regions including Fukuoka Pref. Figure 1 is the map showing the distribution of wild *L. speciosum* in Japan. The habitats in Ehime and Kumamoto Prefs. and the mainland of Kagoshima Pref. may be questionable, but the last two would probably be situated in the area containing the past habitats around the Islands of Koshiki. In Kyûshû, they are found in the western coastal district ; and in Shikoku, mainly in the south-eastern interior.

The nature of the ground in the habitats is the Palaeozoic strata and sometimes the Mesozoic strata in Shikoku ; and in Kyushu, the Mesozoic strata, the Tertiary

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strata and the igneous rocks. Their soils are generally clayey. The lily grows on sloping grass lands and cannot be found in forests, because sunshine is necessary for its growth. The plant usually found with it, is *Miscanthus sinensis* Anderss. The environment of the habitat differs regionally. In Kôchi and Tokushima, it is a steep slope with grass or a cliff, surrounded by the evergreen forest in the valley of 50 to 159 meters above the sea-level. In Nagasaki, it is generally a grass-step girdling a rock above the sea and the rock has a evergreen wood on the tip. Although there are rock cliffs on the seaside of the Islands of Koshiki, the habitat is a sloping and sunny grass land containing *Miscanthus sinensis, Pteridium aquilinum* Kuhn and others. In the Isle of Taira, one of the islands, the most dense colony of the lily can be found in the grass, covering almost the whole island. Although the altitude of the habitats is generally near the sea-level, it sometimes attaining to 300 meters in Koshiki, and as high as 800 meters in Kôchi.
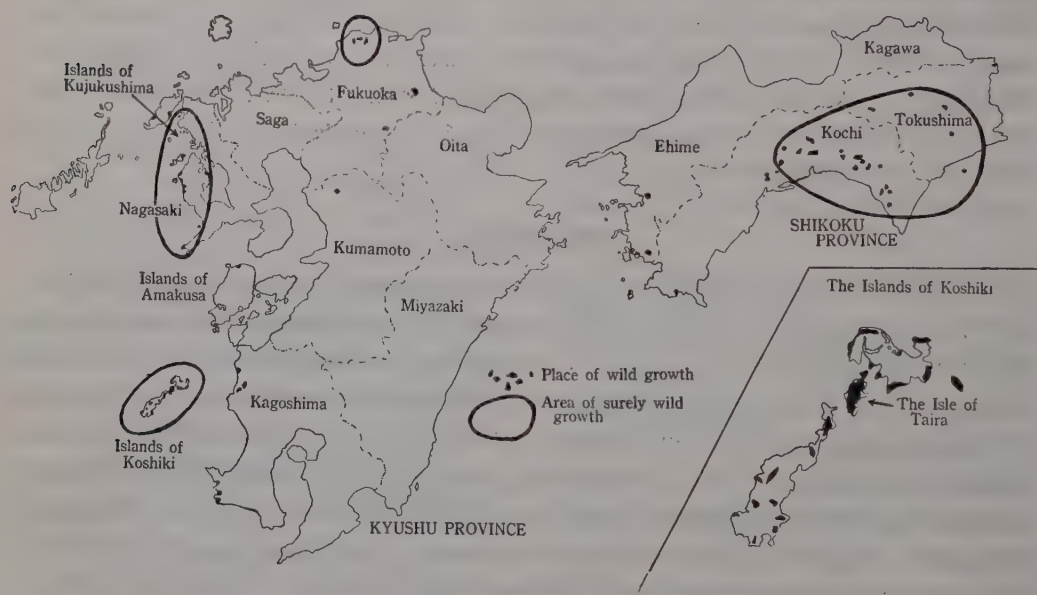

Fig. 1. Distribution of wild plants of *Lilium speciosum* in Japan.

Characteristics and Variations

The wild bulbs collected from different regions were set and cultivated in the field of this station in Kurume in 1952 and 1953 to observe their characteristics. The bulbs set in 1952 were grown for two succesive years, in order to examine the change of their characteristics.

Bulb—The bulbs show considerable variations in their shape, especially in their shade. The shades vary between reddish purple and brownish yellow. Reddish tint is variable and is not same by years. It tends to become deeper after the bulb was dug out, and the bulbs from the Koshiki- and Formosa-plants become gene-

rally reddish purple or red after digging. The bulbs have bitter taste in various degrees.

Stem—Length of the stem is various and different according to ecological condition. It becomes longer in a forest or if surrounded by tall grasses, and shorter in a sunny and dry place. Shades of the stem are distinguished into purplish brown, light brown and green. The shade varies according to the region of origin.
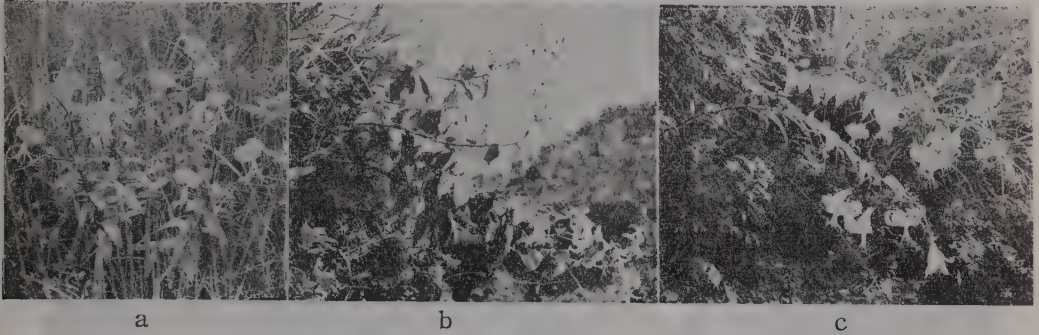

a　　　　　　　　　　　b　　　　　　　　　　　c

Fig. 2.　Habitats of wild *Lilium speciosum*.
　　a.　The Islands of Koshiki, Kagoshima Pref.
　　b.　The Islands of Kujukushima, Nagasaki Pref.
　　c.　A valley of Kôchi Pref.

Form of plants—Forms of the plant in the wild habitat are various from erect to sub-pendulous, according to environmental factor and to the growing region; in Koshiki Islands, erect plants are mainly found (Fig 2a), but sometimes they become sub-erect, while in Nagasaki (Fig. 2b) and Kôchi (Fig. 2c) the plants are sub-erect, sometimes sub-pendulous. The form also differs according to the age of the plant; yong plants are generally sub-erect, but become erect as they become old. The young plants are sub-erect even in the Koshiki Islands and the sub-pendulous plants in Kôchi are generally found to be young ones having only 1 or 2 flowers. In the case of the plants cultivated under the same condition, the more the leaves numerous, the more the stem erect; but concerning the condition of the lower part of stems of the aged plants from various places, no distinct tendency could be recognized. But the condition of the upper part of stems seems to be controlled by other factors, because there are sometimes found the cases in which the lower part is erect and the upper part is nodding (Fig. 3g), or, the cases in which the lower part is sub-erect and the upper part is rising (Fig. 3b). The nodding character of a stem is peculiar to the plants from Kôchi and Tokushima. It is probably due to thin and slender nature of the stem. The nature of the lower stem seems to be more controlled by environmental factors than ly hereditary ones. The nodding character of a stem is clearly seen in the wild plants cf Kôchi (Fig. 2c) and Tokushima.

Leaves—The phyllotaxis is 2/5, 3/8 and 1/2. Sometimes more than two kinds of the phyllotaxis are observed on the same stem. The phyllotaxis of the upper
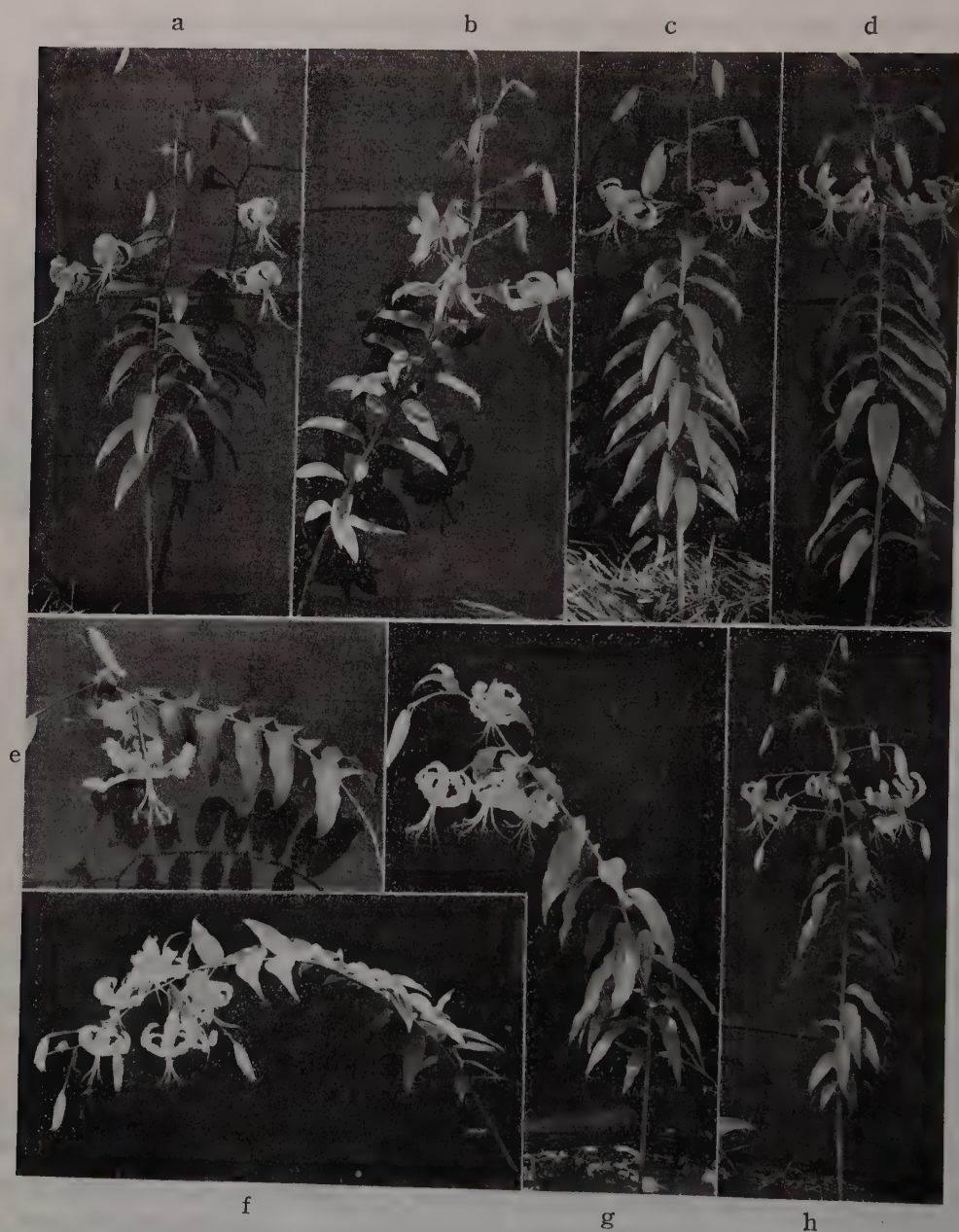
Fig. 3. Cultivated forms from different origins.

a.　　　Formosa.
b—c.　The Islands of Koshiki.
d—e.　Nagasaki.
f—h.　Kôchi.

part just below the flowering portion tends to become 1/2. The 1/2 phyllotaxis is usually found on young plants and tends to disappear as they become old. The frequency of appearance of the 1/2 phyllotaxis differs according to the origin, and it

appears less on the plants from Koshiki. The 1/2 phyllotaxis has a close relationship to the form of a plant. The plane arrangement of leaves is a favorable condition for receiving sunlight in the sub-erect stemmed plant. The erect plant with the 1/2 phyllotaxis twists its stem in order to avoid the plane arrangement of leaf blades, and on the other hand, the sub-erect plant with the 2/5 or 3/8 phyllotaxis tends to arrange its leaves flatwise. Accordingly, the 1/2 phyllotaxis can be considered as a case of the adaptive variation appeared in a group which grows on the cliff and whose stems are apt to become sub-erect.

The direction of leaf blade varies from pendulous state to widely patent state, and differs according to the region. The leaves are various in shape from elliptic to lanceolate. The shape of tip of the leaves is acute or attenuate. The length
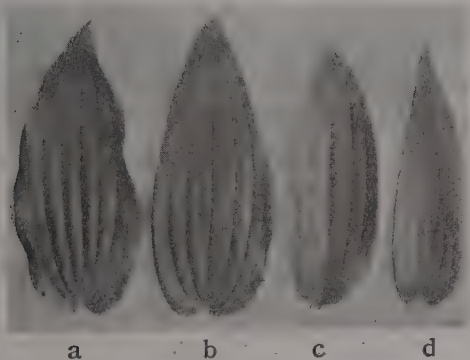

Fig. 4. Variations in impression along veins
and undulation of margin in leaves.

a. Kôchi–origin.   c. Koshiki–origin.
b. Nagasaki–origin.   d. Formosa-origin.

Fig. 5. Shape of stigmata from
different origins.

a. Formosa.
b. The Islands of Koshiki.
c. Nagasaki and Kôchi.

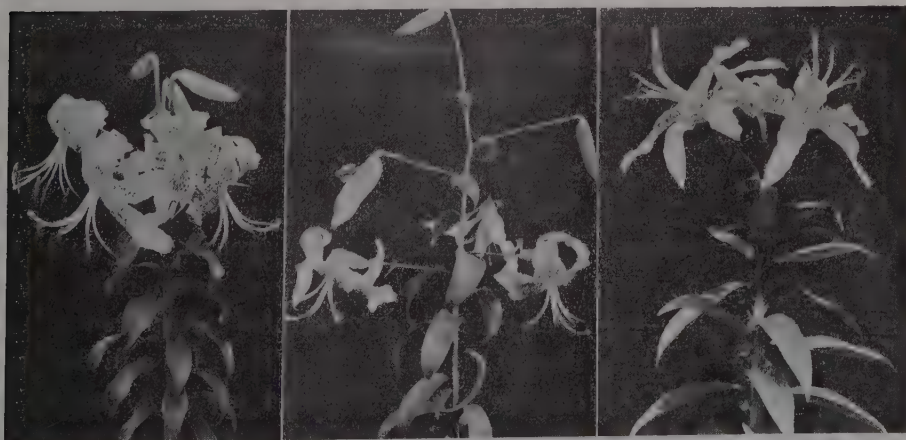Fig. 6. Variation in length of pedicels.
left: the short pediceled form
(f. *compactum* S. Abe et Tamura).
right: the longer pediceled one.

Fig. 7. An example of sub-erect
direction of flowering (f.
erectum Walker).

and thickness of leaves show continuous variation. Shades of a petiole are purplish brown, light brown and green, like those of the stem. Shades of the leaf blade vary between rather dark green and yellowish green. These characters, leaf shapes, and length, thickness and shades of blade correlate in difference with the region of the plant. The leaf blade is impressed along veins, the surface waving in various degrees. The margin of leaves also undulates variously or does not at all, and the undulating character differs according to the region (Fig. 4).

Flower—The length and thickness of a pedicel vary highly and continuously (Fig. 6), and differ according to the origin.

All the flower buds are erect in direction, until their size attains about 2 cm., but they turn their directions variously before flowering. The direction of flowering varies between pendulous one to sub-erect one (Fig. 7), and is rarely erect. It is affected by the internal condition, and apt to become more erect in younger plants from Koshiki. In the plants from Kôchi and Tokushima, pendulous direction of flowering is frequently seen (Fig. 3f, 3g). It seems to be due to slenderness of their pedicels, but, seeing that all capsules take erect position, the pendulous direction of flowering may not be affected by the mechanical condition of pedicels.

The shape of perianth-segments varies between lanceolate and elliptic, but the difference according to the origin cannot be recognized. The perianth-segments reflex variously and very rarely does not at all. They wave variously in their margin. These two characters differs according to the origin.

In the size of the flower, considerable variation is recognized. The flowers of the plants from Koshiki and Nagasaki are large, ane those from Kôchi, Tokushima and Formosa are rather smaller. Factors which decide the size of the flowers of *L. speciosum* are the size and the degree of reflexing of perianth-segments. Taking the size into no account, the flowers looks largest when the reflexibility of perianth-segments is slight and they are arranged in plane. The size of perianth-segments does not differ according to the origin except that the size of the plants from Formosa is smaller. The small appearance of the flowers of the plants from Kôchi and Tokushima is due to high reflexing of the perianth-segements.

The perianth-segments are reddish, especially at the part about one-third distant from their base, and they are very rarely white. The perianth-segments are of ground color and spotting. The grade of deepness of ground color varies highly and continuously, from crimson to pink especially from rose-carmine to rose-pink. There are distinguished many types of coloring, concerning the position and extent of coloring, the state of fading toward the margin of colored part and the presence or absence of spotting. These coloring may be classified into eight types; entire, margined, faded, clotched, rayed, unspotted, white-grounded, and white-eyed, as shown in Figure 8. There are also recognized intermediate types of coloring, only between margined or faded and blotched or rayed, for example, the margined and blotched. The entire and the rayed are not frequently found, and the white-grounded,

unspotted and white-eyed are very rare.

The shades of spots vary continuously from bordeaux to rose-pink, especially from dark crimson to rose. The sizes of spots range from less than 1.5 mm. to over 3.5 mm., and their number ranges from less than 20 to over 60 per segment (inner). The shades of nectariferous furrows can be distinguished into green, light green, yellowish green, greenish yellow and light yellow. Those of the plants from Formosa are green, but in the plants of other origins, no difference could be recognized.
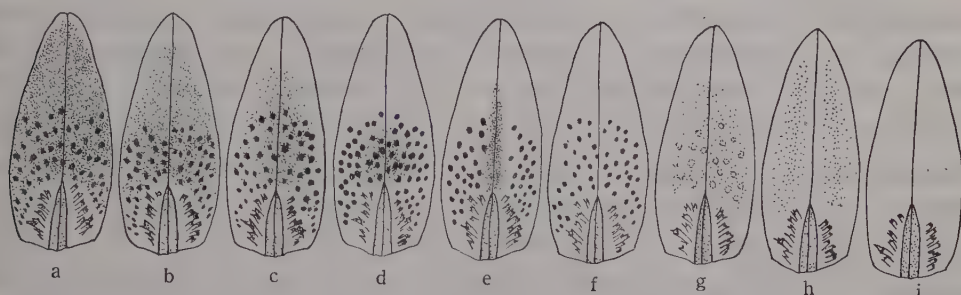

Fig. 8. Type of coloring of flower-segments.

a. Entire. d. Blotched. g. White-eyed.
b. Margined. e. Rayed. h. Unspotted.
c. Faded. f. White-grounded. i. Unspotted (white).

The color of pollen is usually chocolate brown, sometimes orange-brown and very rarely yellow. Yellow pollen has been found so far only in the flowers whose ground color is white. It occurs sometimes in the reddish flowers of field-grown plants, but this is considered to be induced by virus diseases. Sometimes both brownish and yellow pollens are found in the same plant, or even in the same flower. The rate of germination of the yellow pollen, considered as abnormal, has been found to be lower than the normal yellow pollen (f. *punctatum*), as the result of the germination test.

The shape of a stigma differs distinctly according to the origin, as shown in Figure 5. The stigmata of the plants from Nagasaki, Kôchi, and Tokushima are sub-capitately truncate, those from Koshiki are capitate, and those from Formosa are sub-capitately truncate and sub-constrict. The colors of the stigma are distinguished into white, green, faint purple, purple and dark purple. There has been found no consistent difference. The capsules of the Koshiki-plants are a little larger than those of the Nagasaki- and Kôchi-plants, while in the Formosa-plants, somewhat smaller.

The time of flowering differs highly according to the origin, when they are grown in the same condition.

Correlation between various characteristics—Among various characteristics, correlation could be found in the following combinations, i. e., between the deepness

Table 1. Comparison of characteristics of *Lilium speciosum* from different origins

Type / Origin / Characteristics	I — Formosa	II — Kagoshima Kumamoto Fukuoka	IIIa — Nagasaki	IIIb–b′ — Kôchi (IIIb) Tokushima (IIIb′)
Bulb:				
Whole shape	flattened globose	rather flattened globose to globose	rather flattened globose	flattened globose
Shape of scale	lanceolate-ovate	lanceolate-ovate	broad lanceolate	lanceolate
Bitterness	strong	strong	slight	slight
Stem:				
Length	considerably long	short	considerably long	long (IIIb) short (IIIb′)
Relative thickness	intermediate	thick	intermediate	thin
Shade	purplish brown	usually light brown	usually light brown	usually green
Leaves:				
Phyllotaxis	tending to 1/2	less tending to 1/2	tending to 1/2	tending to 1/2
Direction	lateral to sub-pendulous	usually lateral	lateral to sub-pendulous	usually pendulous
Shape	lanceolate to oblong-ovate	lanceolate to oblong-ovate	broad lanceolate to elliptic-ovate	broad lanceolate to elliptic-ovate
Size	——	——	large	large (IIIb) small (IIIb′)
Thickness	very thick	thick	intermediate	thin
Deepness of shade	medium	rather pale	medium	deep
Impression along veins	very slight	slight	strong	strong
Tip	usually acute	usually acute	usually attenuate	usually attenuate
Undulation in the margin	very slight	very slight	strong	strong
Flowers:				
Pedicel	long and slender	short and thick	intermediate	long and slender
Direction	sub-pendulous	sub-pendulous	sub-pendulous	pendulous
Reflexibility	usually strong	usually slight	intermediate	usually strong
Undulation in the margin	rather strong	usually slight	usually slight	rather strong
Deepness of shade	usually deep	usually deep	intermediate	usually pale
Type of coloring	blotched or white-grounded	usually margined	usually blotched	usually faded
Style	short	long	intermediate	intermediate
Ovule	somewhat small	somewhat large	——	——
Stigma	sub-capitately truncate. sub-constrict	capitate	sub-capitately truncate	sub-capitately truncate
Time of flowering	late Aug. to early Sept.	late July to early Aug.	late July	early to middle July (IIIb) late June to early July (IIIb′)
Fruit	rather small	rather large	——	——

of ground color and that of a flower, as well as between the deepness of ground color and the type of coloring of a flower. In the former case, the deeper the ground color is, the deeper the shade of spots is; and in the latter case, the larger the extent of color, the deeper the ground color.

Regional differences—Table 1 is a summary of characteristics of the plants from different origins. The shape of a stigma, the feature of the tip of and the impression along veins of leaves are the most distinguishable characteristics. In these respects the plants from Formosa, those from Koshiki and those from Nagasaki, Kôchi aud Tokushima show characteirstics that can be easily distinguished. In the last group, there could be found considerable differences between the Kôchi-, Tokushima-plants and Nagasaki-plants concerning the following: the form of the plant (the state of the upper stem),—especially whether it is apt to become pendulous or not—the direction and thickness of leaves, the direction of flowering, the reflexibility of perianth-segments, the time of flowering and other less remarkable characteristics. Thus four groups can be discriminated. The variations of characteristics of each group are sometimes remarkable and usually overlap each other, but any plant of each group shows fixed type as a whole, even if grown in the field under the same condition. Wild habitats are regionally isolated each other, and therefore groups above mentioned may be considered respectively to be geographical races. The race of Nagasaki and that of Kochi and Tokushima resemble more or less one another, but the former is of coastal origin and the latter is of inland origin. The plants from Jinryô village, Tokushima Pref. are a little smaller in size than those from Kôchi. Thus, the geographical races of *L. speciosum* are to be divided into four types, i. e., Formosa-type (Type I), Koshiki-type (Type II), Nagasaki-type (Type IIIa) and Kôchi-type (Type IIIb). From the last type, the Jinryô group may be distinguished and named as Type IIIb′. It is found that the plants from the mainland of Kagoshima Pref., Kumamoto Pref. and Fukuoka Pref. in Kyûshû Province, and those from Ehime Pref. and the western part of Kôchi Pref. in Shikoku Province belong to the Koshiki-type. However, it is doubtful whether the Koshiki-type of Shikoku Province is spontaneous or not. Concerning the degree of the variation, the plants of Koshiki-type seem to be most intense among these four geographical races. The Koshiki-type is also the strongest and the easiest for cultivation.   (*To be continued*)

The Growth of Rice Leaf Sheath

by Yutaka MURAKAMI

村上 浩: イ ネ 葉 鞘 の 伸 長

It has been known that the growth of isolated coleoptile sections is promoted by auxins. The growth of leaf sheath sections from green grasses is less understood than that of coleoptile sections. Unlike the coleoptile, which reacts sensitively to light, the green leaves of grasses do not bend towards the source of the light. Hence, it seemed interesting to examine whether or not the growth of the green leaf sheath sections of grasses is due predominantly to auxin or to other factors. The present work deals with the leaf sheath sections isolated from the sixth growing leaves of young rice plants.

Material and Methods

Rice plants (var. Aichi-Asahi) were grown in summer and used in all the experiments described in this paper. The seeds were selected from the stock and immersed in 0.1 % " Uspulun " solution for 20 minutes. They were then washed thoroughly and permitted to germinate in running water. When the emerging coleoptiles attained about 1 mm in length, the seedlings of the same size as far as possible, were selected and placed in the wet seed-bed with complete nutrient. At the age of about 20 days, when they reached a length of about 30 cm, plants started to develop the sixth leaf. In such plants active growth is largely restricted to this sixth leaf sheath. Plants were selected whose sixth leaf had attained a length of about 18 cm. At that stage the sheath of the sixth leaf was about 20 mm in length and ready for use. For use in most of the present experiments the leaf sheath sections of 3–5 mm long were cut with a double-bladed cutter at a distance of 1 mm from the base of the sheath (see Fig. 1).

Length measurments.   After the selection of plants the sixth leaves were gathered. The experimental sections were excised and placed in glass-distilled water. Random samples of 15 sections were then immersed in 25 ml wide mouth bottles containing 5 ml of the culture medium. The bottles were placed in the dark in an incubator at 30°C. Length measurements were made after 20 hours with an ocular micrometer equipped in a stereoscopic binocular dissecting microscope.

Sections sometimes exhibited contorted growth, making the length measurement difficult.

Auxin determinations.   Amounts of auxin were determined by the method of diffusion[1] and extraction[2]. Diffusion method: The sixth leaf sheath of 20 mm in length was divided into an upper and lower half. The cut sections were placed with their bases on agar blocks, twelve sections per twelve blocks, and kept for 3 hours. The blooks were then tested on the *Avena* coleoptile. Extraction method: 200 sections from upper and basal regions, each 8 mm long, were extracted respectively with ether. Ether was evaporated and the residue was mixed with 0.2 ml of 2 % agar. The agar was cut into twelve small blocks in the manner described by Van Overbeek[2] and the blocks were then tested on *Avena* coleoptile.
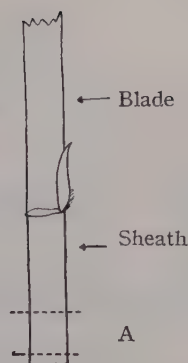

Fig. 1. The lower portion of the sixth leaf of a rice plant. A. Portion utilized for section growth experiments.

Results and Discussion

The preliminary tests of the growth of green sheath sections showed that practically no elongation occurred in the absence of sucrose. Representative results from such growth tests are shown in Fig. 2. The optimum sucrose concentration appears to be in a range of 2–4 %. Glucose and fructose possessed activities equal to that of sucrose.

Experiments were next conducted to make clear the nature of a factor or factors which, in the presence of sucrose, limit the growth in length of the leaf sheath section. In order to screen compounds for their possible effects on the growth of leaf sheath sections, the test materials were incorporated into basic media containing 2 % sucrose in 1/200 M phosphate buffer of pH 5.5. For the

Fig. 2. The effect of sucrose concentration on growth of rice leaf sheath sections. Initial length of section was 5 mm.

tests, inorganic salts and organic acids were added at concentrations of 100 and 200 μg/ml. Amino acids, vitamins, and growth hormones were tested at concentrations ranging from 0.1 to 100 μg/ml, except for growth hormones which were added at concentrations of 0.1 and 1 μg/ml.

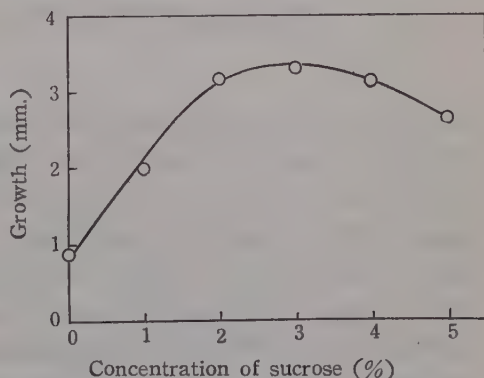The results are summarized in Table 1. $MnSO_4$ and succinic acid stimulated

Table 1. The effect of various compounds on the growth of leaf sheath sections
excised from rice plants.

Class of compounds	Compounds tested	Concentrations employed μg./ml.	Growth effect
Inorganic salts	KCl, KNO$_3$. CaCl$_2$. MgSO$_4$. NH$_4$Cl, MnSO$_4$, FeSO$_4$, CoCl$_2$.	100 & 200	Slight growth inhibition by CaCl$_2$; about 15% growth stimulation by MnSO$_4$.
Organic acids	Fumaric, succinic, malic, citric, pyruvic, and α-ketoglutaric acids.	100 & 200	About 15% growth stimulation by succinic acid.
Amino acids and amide	DL–α-Alanine, β-alanine. L–arginine. L–aspartic acid, L–cystine, L–glutamic acid, glycine, L–histidine HCl, L–hydroxyproline. L–isoleucine, L–leucine, DL–lysine HCl, DL–methionine, DL–β-phenylalanine, DL–proline, DL–serine, L–tryptophan, L–tyrosine, DL–valine, asparagine.	0. 1-100	Slight inhibition by DL methionine and DL–β-phenylalanine.
Vitamins	Thiamine HCl, lactoflavine, pyridoxine HCl, nicotinamide, Ca pantothenate, biotin, adenine sulfate.	0. 1-100	Slight inhibition by nicotinamide and adenine sulfate.
Growth hormones	Indoleacetic(IAA), naphthalene-acetic(NAA), phenylacetic(PAA), and 2, 4–dichlorophenoxyacetic acids(2, 4–D). 2-Chlorophenoxyisobutyric and 4-chlorophenoxyisobutyric acids. 2, 3, 5–Triiodobenzoic acid. Gibberellin.	0. 1 & 1	Marked inhibition by IAA, NAA, PAA. and 2, 4–D. About 30% growth stimulation by gibberellin.

the growth. Gibberellin exerted a considerable stimulation of growth. CaCl$_2$, DL-β-phenylalanine, nicotinamide and adeninesulfate were slightly inhibitory. Auxins such as indoleacetic acid (IAA) completely inhibited the growth of sheath sections. Other compounds were found to have no detectable effect. It is a very curious feature that the sheath section growth was powerfully inhibited by auxins. The activity of IAA was then further investigated over a wide concentration range, and the effects of IAA and phenylacetic acid(PAA) are summarized in Fig. 3. In a solution of 0.1 μg/ml IAA, the growth of sheath sections was almost completely inhibited. With decreasing concentration of IAA the inhibition of the growth reduced, and concentrations as low as 0.0005 μg/ml caused no measurable effect. PAA, which possesses extremely low activity in promoting the excised coleoptile sections[3], was less inhibitory than IAA. No increase of the section growth even in low concentrations of IAA or PAA was observed in these experiments. α-Substituted isobutyric acids, which are inactive in the coleoptile section test[4], were also inactive in the leaf sheath section. These facts are taken to indicate that the chemical structure which is required for the growth activity on coleoptile growth is almost similar to

that which is responsible for the growth inhibition of the leaf sheath. This suggests also that auxin is involved in an initial reaction which is common to sheath inhibition and coleoptile elongation.

Fig. 3. The effect of auxin concentration on growth of rice leaf sheath sections. Initial length of sections was 5 mm. (○) IAA, (×) PAA, A: growth in distilled water.

Since cells in the sheath are continuously being formed at the base and grow immediately as they are formed, the successive sections contain cells of more advanced stages of development. In a subsequent experiment, four successive 3.24 mm sections of the sheath were cut in a five-bladed cutter. Each section was placed in a separate bottle the sections being labelled No. 1, 2, 3 and 4 from the base of the sheath. Then they were allowed to grow as indicated above. The results are represented graphically in Fig. 4. The data show that IAA was not stimulatory to the elongation of the sections obtained from upper and middle as well as basal regions. The basal sections labelled No. 1 showed the most inhibitory response to IAA, and they grew most vigorously in the absence of IAA. The growth inhibition by IAA decreased from the basal to the upper sections and the growth rate of each section also showed just the same trend. Even higher concerntrations of IAA could not cause the elongation of sections No. 4, which did not grow in sucrose.

From these observations auxin distribution in the sheath appears to be of importance for the understanding of the growth of the sheath sections. Hence, determinations of the auxin content of basal and upper regions within the sheath were
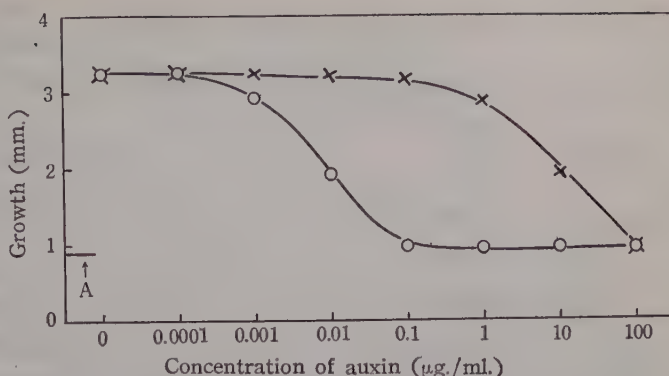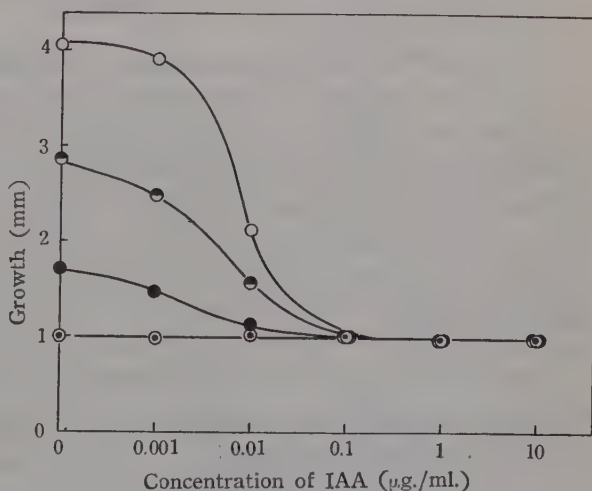

Fig. 4. The effect of IAA concentration on growth of sections excised from different regions of rice leaf sheath. Distance from base of leaf sheath (mm.): No. 1 (○) 1-4. 24, No. 2 (◐) 4. 24-7.48, No. 3 (●) 7.48-10.72, No. 4 (◉) 10. 72-13. 96.

Table 2.　Auxin assay of leaf sheath sections

Method	Region	Degrees of curvature
Diffusion	upper	0.0
	basal	0.0
Extraction	upper	− 7.1±0.7
	basal	−18.0±1.6

Standard curvature produced by IAA (50 μg./l.):
−18.1±0.86

made using the method of diffusion and extraction.　Table 2 gives the result of these estimations. The free moving auxin, which can be demonstrated by the diffusion method, was not found in the sheath.　On the other hand, the bound auxin, which is obtained by the extraction with ether, was present all over the sheath.　The basal regions of the sheath, in which the growth was inhibited by exogenous auxins, contained much more bound auxin than the upper regions.

　　To account for the growth distribution in etiolated seedlings of grasses, Went[5] proposed the so-called two-factor scheme.　This scheme is based on the interaction of two factors both necessary for growth.　Auxin, one of the factors, is produced at the tip of the coleoptile and transported downward.　The other factor is called the "food factor" which is supplied by the seed.　As shown in Table 2, a relatively high auxin concentration was found at the basal regions of the sheath, decreasing towards the upper region, a distribution quite the reverse of the coleoptile.　Fig. 3 also shows that sections from basal regions grow vigorously, if supplied with sucrose. According to the two-factor scheme, it may be tentatively explained that the growth of the basal regions of the sheath is limited by the food factor.

Summary

　　The growth of the green leaf sheath sections excised from young rice leaves occurred in the presence of the sugar such as sucrose.　Inorganic salts, organic acids including amino acids, vitamins, and growth hormones were tested for their effects upon the growth of the sections in the presence of 2 % sucrose.　Auxins inhibited the sucrose-induced growth of the leaf sheath sections at the concentrations that stimulate the growth of coleoptile sections.　The growth of the basal tissue of the leaf sheath, where auxin was in excess, seems to be limited by nutrients other than auxin.

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Studies on a Polyphenolase in *Scopolia japonica* II
On the Oxidation of Monohydric Phenols

by Yonezô SUZUKI*

鈴木米三*： ハシリドコロのポリフェノラーゼの研究　II. モノフェノールの酸化について

In the previous paper[1] the author showed that the polyphenolase from the subterranean stem of *Scopolia japonica* was not able to oxidize monohydric phenols directly. While, it is well known[2] that the induction period of the polyphenoloxidase acting upon various monohydric phenols can be eliminated if a small amount of a reducing agent or a diphenol is added. The method and result of experiments carried out in respect of the problem now in question are described.

Material and Method

The crude polyphenolase (15.0 mg. dry weight per 1.0 ml.) was prepared, from the subterranean stem of *Scopolia japonica*, by the method as described in the previous paper[1]. Oxygen uptake was determined manometrically at 35°C and pH 7.3 L-ascorbic acid or dopa (each 0.5 ml.) being contained in a side arm, while the enzyme solution (1.0 ml.) was contained in a main chamber. The extent of the oxidation of *p*-cresol was determined with an electric colorimeter using 480mμ filter.

Results and Discussion

1. Oxidation of monohydric phenols in a longer period of reaction.

It is shown in Table I that all the phenols except L-tyrosine were oxidized considerably by the polyphenolase of *Scopolia japonica* in 20 hours, showing a colour development in different degrees. L-Tyrosine was, however, readily oxidized by the polyphenolase prepared from the potato tuber exactly in the same way. The polyphenolase of *Scopolia* has in some respects a like property of an oxidase in *Stizolobium hassjoo*, reported recently by Hattori and Shiroya[3] as the *Stizolobium* enzyme did not oxidize the L-tyrosine.

2. Effects of dopa and L-ascorbic acid on the oxidation of monohydric phenols.

As shown in Fig. 1 and 2, the addition of a small amount of L-ascorbic acid as reducing agent or dopa as diphenol, shortened remarkably the induction period in the oxidation of *p*-cresol and phenol.

It might also be suspected that the addition of the reducing agent or the diphenol caused only seemingly but not truly the increase of oxygen uptake by

* Biological Institute, Toyama University, Toyama.　富山大学文理学部生物学教室

Table I.　The oxidation of monophenols by polyphenolases.

Phenols	polyphenolase from *Scopolia japonica*	polyphenolase from potato tuber
catechol	++	
dopa	++	
p-cresol	++	+++
phenol	+(+)	
L-tyrosine	−	++

Remarks: 1)　The oxidation was detected by the colour development in the mixture containing 2.0 ml. of enzyme solution, 2.0 ml. of phosphate buffer (M/15, pH 7.3) and 1.0 ml. of substrate solution, after 20 hours of reaction at 35°C.
2)　Each control tubes were made of 2.0 ml. of dist. water, 2.0 ml. of phosphate buffer (pH 7.3) and 1.0 ml. of substrate solution.
3)　Concentrations of substrate solutions: catechol 10^{-2}M., DL-dopa 10^{-3}M., *p*-cresol 0.032M., Phenol 0.032 M. and L-tyrosine suspension.

the monohydric phenols themselves. It is shown, however, clearly in Table II that the increase in oxygen uptake is always accompanied with an increase in the optical density of the resultant solution. Thus the monophenols have certainly been oxidized by thd polyphenolase, as was described in the former work. Similar results have also been obtained with an insoluble and a soluble enzyme separated from the leaf of this plant[4].
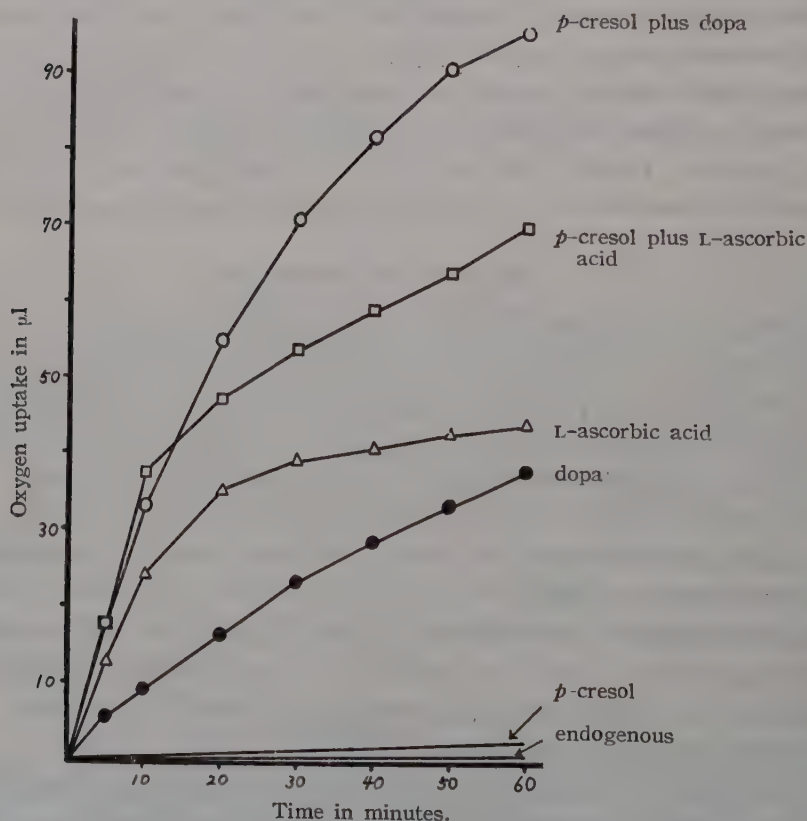

Fig. 1.　Effects of dopa and L-ascorbic acid on the oxidation of *p*-cresol.
(Final concentrations: DL-dopa 0.25×10^{-3} M., *p*-cresol 0.008M., L-ascorbic acid 1.25×10^{-3} M.).
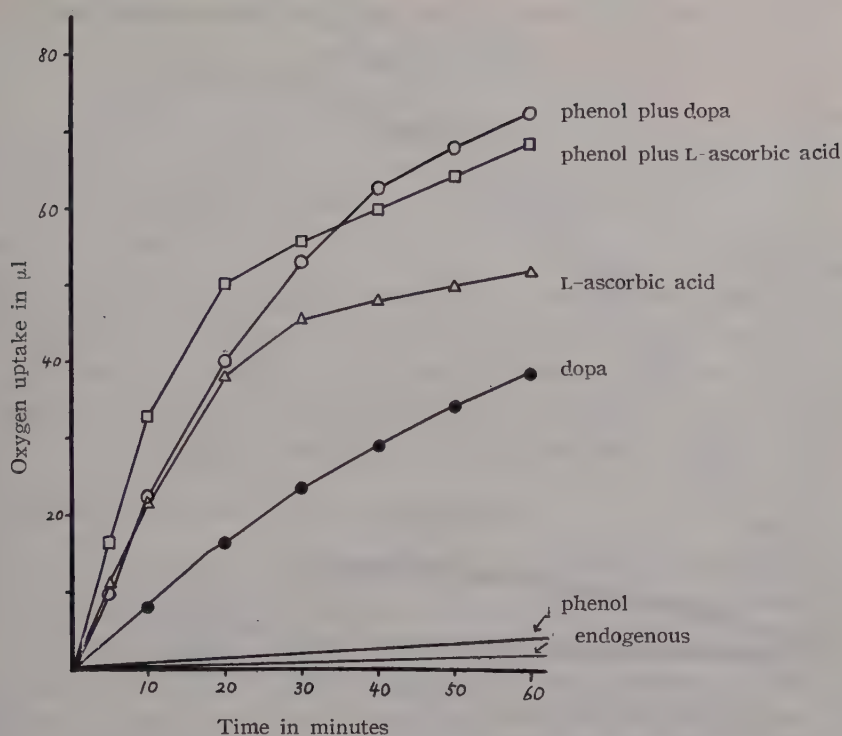
Fig. 2. Effects of dopa and L-ascorbic acid on the oxidation of phenol.
(Final concentrations: DL-dopa 0.25×10^{-3} M., L-ascorbic acid 1.25×10^{-3} M.,
phenol 0.008M.).

Table II. Effects of L-ascorbic acid and dopa upon the oxidation of p-cresol
by means of polyphenolase.

Exp. No.	p-cresol	polyphenolase from *S. japonica*	L-ascorbic acid	DL-dopa	optical density* $(-\log T)$	O_2-uptake µl
I.	3.5 mg.	1.0 ml.	0.8 mg.	—	0.17	75 (in 60 min.)
II.	—	1.0 ml.	0.8 mg.	—	0.01	45 (in 60 min.)
III.	3.5 mg.	1.0 ml.	—	—	0.01	0.3 (in 60 min.)
IV.	3.5 mg.	1.0 ml.	—	0.1 mg.	0.32	44.3 (in 30 min.)
V.	—	1.0 ml.	—	0.1 mg.	0.16	13.3 (in 30 min.)
VI. (cont.)	—	1.0 ml.	—	—	0.00	0.4 (in 60 min.)

Remarks: Total volume of reaction mixture was made up to 4.0 ml. with 1.0 ml. of phosphate
buffer (M/15, pH 7.3) and distilled water.
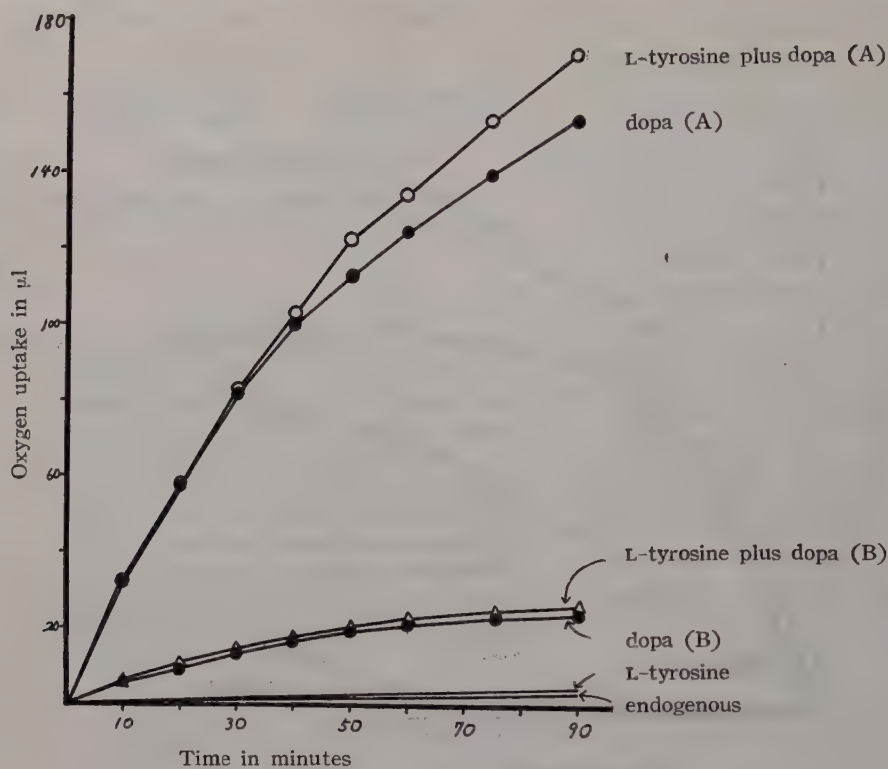* Reaction mixture was diluted to twice volume with distilled water.

Fig. 3. Effect of dopa on the oxidation of L-tyrosine.
(Final concentrations: L-tyrosine suspension, DL-dopa 1.25×10^{-3}M. (A),
1.25×10^{-4}M. (B)).

On the other hand, neither the L-ascorbic acid nor the dopa was effective upon the oxidation of L-tyrosine. The result is shown typically in Fig. 3. Table I should also be cited.

Summary

1. It was shown that a crude polyphenolase from the subterranean stem of *Scopolia japonica* oxidizes, in a longer period, *p*-cresol and phenol but not L-tyrosine.

2. L-Ascorbic acid and dopa shorten remarkably the induction period in the oxidation of *p*-cresol and phenol; but they are not effective upon the oxidation of L-tyrosine.

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On some Abnormalities in Japanese Species of the Ophioglossaceae*

by Yoshitomo Nozu

野津良知： 日本産ハナヤスリ科植物の奇形について

Studies on the abnormalities in the Ophioglossaceae have been carried out by Roeper (1859) in *Botrychium obliquum*, by Goebel (1884) in *B. Lunaria*, by Bower (1896, 1908, 1911) in a few species of *Ophioglossum*, by Chrysler (1910, 1925, 1926) in *B. lanuginosum* and *B. obliquum* and also by others. Here the writer deals with observations on some abnormalities of Japanese species of this family.

Observations

A. Forking or duplication of the fertile spike is the most interesting abnormality of the Ophioglossaceae and it may be roughly divided into two different types.

a) One type is shown by the forking or duplication of the normal fertile spike itself. Typical examples of this case are found in *B. ternatum*, *O. vulgatum* and *O. ellipticum* (Fig. 1, A, F). In *B. ternatum*, forking occurs at the base of stalk of the fertile spike, while in *O. vulgatum* and *O. ellipticum* at the upper part of the stalk or at the sporangious region. In order to know the course of the bundles, serial sections at the forking region are traced. In *B. ternatum*, the O-shaped vascular bundle** below the branching level divides into two unequal parts in the tangential plane (Fig. 2, A). The smaller C-shaped bundle enters into the fertile part, while the larger one into the sterile part, and the former again divides more or less equally, in a plane perpendicular to the preceeding division and then each bundle divides again into two equal parts. Each of two bundles soon appears like a pair of parentheses and enters into two fertile segments. The same condition is observed in *B. japonicum*, *B. Lunaria* and *O. pendulum* (Fig. 3).

In *O. vulgatum* and *O. ellipticum*, there are four large or small bundles arranged in a plane at the top of the fertile stalk. At a short distance below the branching level, the larger one of the four is divided into two which enter into the branching. Such a forking is also observed in other species of *Euophioglossum* and *Ophioderma* of *Ophioglossum* and in the subgenera *Osmundopteris* and *Eubotrychium* of *Botrychium*.

** It was already described by the writer (1955) that the O- or U- shaped vascular bundle occurs at the top of the phyllomophore of *B. ternatum*.
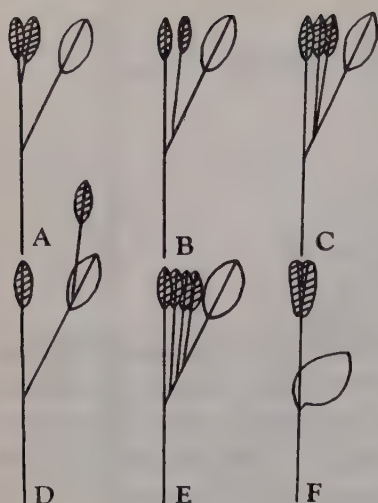
Fig. 1. Diagrams showing the forking or duplication of the fertile spike in abnormal forms of *Botrychium ternatum* and *Ophioglossum ellipticum*. A, the fertile spike equally forking at its top. B, a normal spike, and a smaller spike inserted on the sterile segment. C, a normal spike, and a pair of smaller ones inserted on the sterile segment. D, a normal spike and a fertile spike on the sterile segment. E, normal spike, and three smaller ones inserted on the sterile segment. F, *O. ellipticum* with the fertile spike equally forking at its top.

b) In other type, one or more smaller additional spikes arise at the sterile segment. This type is also shown by *E. ternatum* (Fig. 1, B—E). Branching of the vascular bundles for normal spike and a pair of smaller ones on the sterile part is shown in Fig. 2, B. The O-shaped bundle at the top of the phyllomophore breaks into two unequal pieces. The smaller bundle runs up into the fertile segment. At a short distance further up in the sterile segment, the smaller bundles breaked off from each edge of the C-shaped bundle lead themselves to a smaller additional fertile segment (Fig. 2, B, a). On the other hand a pair of the bundles arises from each edge of the U-shaped bundle of the phyllomophore (Fig. 2, B, b, 2) and enter into the fertile segment (Fig. 2, B, 3). Also a pair of the bundles (Fig. 2, B, 4), as in the preceediug case, divides from each edge of the O-shaped bundle of the sterile part. Two divided bundles rotate so that their xylem groups may come to face the C. These bundles generally approach one another and are soon fused edge by edge at their short distance in their course, but at a higher level they separate again and each bundle divides into two in the radial plane. Then, two bundles on the right hand supply to one spike and other two on the left hand to the other spike. Such a condition is recognized also in *B. Lunaria* and *B. japonicum*. In a few example, however, the
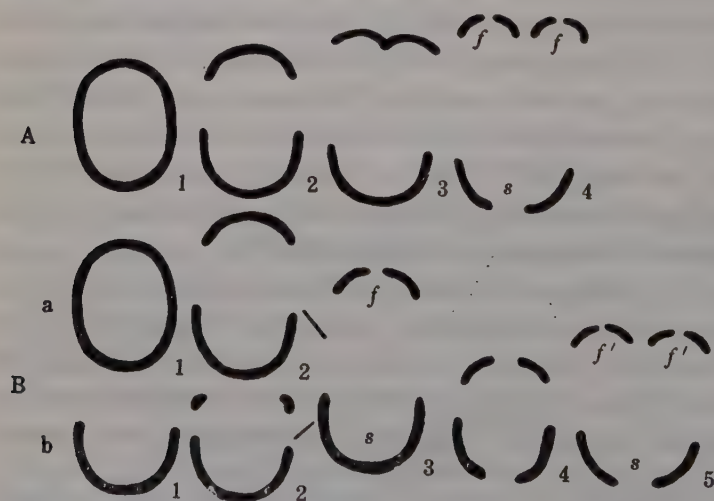

Fig. 2. *Botrychium ternatum*; successive cross sections near the top of phyllomophore, showing the vascular bundles in abnormal fertile spikes. A, the fertile spike equally at its base. B, a normal spike, and a pair of smaller additional ones on the sterile segment ×30. s, sterile segment. f, f' fertile segment.

fertile spike attaches to the base of the sterile segment
(Fig. 1,D) and further, in an extreme case, the fertile
spike arises near the sterile segment. In many cases, addi-
tional spikes are smaller than the normal one.

   B. The fertility of the sterile pinnae is widely distri-
buted among the species of *Botrychium*. As an example
of this case, a part of a basal pinna of *B. japonicum* is
shown (Fig. 4). Usually the sporangia are few in number
and found on the margins of one or more pinnae. In this
case the fertility is acropetal. The features of these
sporangia arise by the partial curing of the leaf margin
similar to that of other modern ferns. This condition is
also found in *B. ternatum*. Very rarely the partial
fertility of the sterile leaf is found in *O. reticulatum*
preserved in the Herbarium of the University of Kyoto
(Fig. 5).

   On the other hand, the transformation of the fertile
spike into the sterile frond is often found in the subgenus

Fig. 3. Abnormal form
of *Ophioglossum pen-
dulum*, showing details
of the additional spike.
× 1/7.
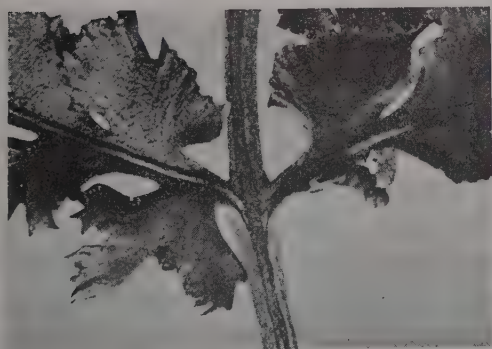
Fig. 4.. *Botrychium japonicum*; sterile
laminae bearing sporangia on the
pinnae. × 1.5.

Fig. 5. Abnormal form of *Ophio-
glossum reticulatum*, showing the
sporangia on a part of the sterile
leaf. nat. size.

Osmundopteris (Fig. 6), especially near the apex of fertile spike, rarely also in the
middle part of sporangious region. Generally, in this case the course is contrary
to the preceeding cases, basipetal.

   C. The variations of sterile segment are of rather rare occurrence in the
sterile segment of *Botrychium*. Normally, the sterile segment of *B. ternatum* has
three principal division. In a few example, however, the basal pinnae show the weak
development. In extreme cases, one of the basal pinna is very incomplete or

entirely deficient. The sterile leaf of *Ophioglossum* shows various degrees of trans-formation of its form. The most common condition is shown in Fig. 7.

Fig. 6. A part of fertile frond of *Botrychium virginianum*, showing a pinnula transformed into sterile one. ×1.5

Fig. 7. Various forms of the sterile leaf of *Ophioglossum ellipticum*, showing details of abnormalities. ×2/3.

Discussion

A. Forking or duplication of the fertile spike was already found by Bower (1908), Chrysler (1910) and others. According to Chry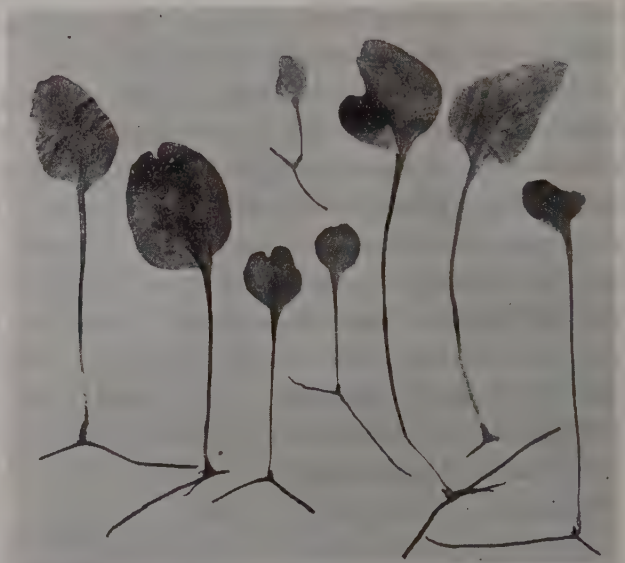sler's observation on *Botrychium*, the highest number of additional fertile spikes is three and they arise from the lateral side of the normal one. In my observation on *B. ternatum* number of them is also from one to three. The position of the additional fertile spikes, however, is not always lateral, but also ventral, and in any case they face to the sterile segment as in the case of normal spike. Usually, the additional fertile spike is smaller than the normal one, but in a few it is similar to that of normal one. It is also a noteworthy fact that the plants showing the additional fertile spike have in most cases O-shaped bundle at the phyllomophore. In my materials the fork-ing of the fertile spike occurs chiefly at the bases of the fertile and the sterile segments. Probably this seems to due to their repeated bifurcations of the bundles in the bases of both segments.

Moreover, it is significant that the additional fertile spike does not attach below the branching part of normal spike. It suggests that the phyllomophore, like the rhizome, becomes more stable than the leaf. In respect of the additional fertile spike at sterile frond Chrysler (1945) observed four cases in *B. lanuginosum*, and in my materials the fertile spike arises near the base or top of the strile frond.

There are seemingly some similalities between the branching in this family

and that of *Anemia*. But in this family the bundles lead to two fertile spikes are derived from O-shaped bundle at the top of the phyllomophore at first as a single bundle, which then splits into two, while in *Anemia* the bundles are derived from each edge of C-shaped bundle, as pointed out by Ogura (1937).

Bower (1926) figured the branching in basal part of the fertile spike of *O. vulgatum* and in sporangial part of the fertile spike of *Helminthostachys zeylanica*. Miyazawa and Yoshie (1938) reported three cases in *B. japonicum*; namely, a pair of the fertile spikes nearly equal in size, a normal spike and a pair of smaller additional ones and a normal spike and the whole fertilization of sterile segment.

B. The occurrence of sporangia on sterile pinnae was observed by many students. Roeper (1859) and Goebel (1884) figured the example in *B. Lunaria*, though the writer has not hitherto seen such an abnormal form in this species. According to Chrysler (1926) this feature is widely distributed among the species of *Botrychium*. Also, Bower (1926) noted that in *Botrychium* the abnormalities are highly variable, and that the distribution of the sporangia is the most important.

Usually, the occurrence of the sporangia is observed in the basal pinnae of the sterile segment, though in extreme cases, the whole lamina may be converted into a fertile organ, as figured by Miyazawa and Yoshie (1938). It is probably due to the fact that a basal pinnae approaches to fertile segment. It may be considered that both sterile and fertile segments of this family are less stable.

The transformation of the fertile spike into the sterile frond has been also recognized by many authors in various species of *Botrychium*. The extent of the transformation may be caused by the plane expansion of the sporangia. Generally, the transformation is apical rather than basal, though in *B. strictum* it is seen at the middle region of the fertile frond.

C. Little has been seen on the nature of the variation of the sterile segment in the Ophioglossaceous ferns. Generally, the abnormality of the sterile segment in *Botrychium* has been hardly seen, though it has been seen rarely, in *B. ternatum*. In *Ophioglossum* the abnormality of the sterile frond is widely distributed. In *H. zeylanica*, Banerji (1951) reports a case in which one of the lateral pinnae is divided again into three.

Summarizing the above facts, the abnormalities can be seen in almost all species of this family, that is, they are the particular tendencies which may be seen in genus or species. Especially, it must be remarked that in these groups the position of the attachment of the additional spike is ventral or lateral, and it shows a tendency moving toward distal direction, that the transformation of fertile spike into sterile frond and the fertilization of sterile leaf are widely distributed among the species like other ferns, and that the ultimate branch of the shoot seems very unstable. From the observation of the various abnormal forms in the family, it is quite clear that this very unstable or plastic fern has an extremely variable primitive character as compared with the other ferns.

Summary

Forking and duplication of the fertile spike are worth noticing abnormalities met in the present study. Forking of the spike was found in *Botrychium ternatum*, *B. japonicum*, *Osmundopteris*, *Eubotrychium*, *Ophioderma* and *Euophioglossum*, and normal fertile spike with additional spikes was found in *Eubotrychium* and *Sceptridium*. The following other abnormalities are recognized. They are the occurrence of sporangia on the frond which is ordinarily sterile (*Botrychium* and *O. reticulatum*), the transformation of the fertile spike into the sterile frond (*Osmundopteris*) and the various malformations of the sterile frond (*Botrychium* and *Ophioglossum*). A detailed account of the situational relationship between normal and additional spikes is given from the view point of vascular anatomy.

In conclusion the writer wishes to express his hearty thanks to Prof. Y. Ogura who gave him many directions, by which the work was carried out. Also, the writer's thanks are due to many others for variable materials.

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　　文部省編　**学 術 用 語 集　植 物 学 篇**　大日本図書株式会社発行，定価 150 円

　上記の植物学用語集が 4 月 25 日に出版された。B 6 版 155 ページで，第 1 部 和英の部，第 2 部 英和の部，第 3 部 植物科名の標準和名の 3 部に分れている。もとより 150 ページの小冊子のことであるから，こまかな専門にわたる用語は採録されていないが，普通のものはたいてい入っているから，十分使用に堪えることと思う。文部省の意図は一般学術用語の混乱をくいとめて整理し，学術用語を平易にして学術の進歩に寄与しようというのであるが，本会もその意をくんで，この用語集の制定編集には全面的に協力してきた。しかし文部省にも，ここにきめた用語を強制する力はなく，またそうしようとする気もないが，植物学者だけでなく，一般の人もできるだけこの用語制定の趣旨に賛成して，大同につくことを希望している。また本会も会員諸氏にこのことをお願いしたい。　　　　　　　　　（服 部 静 夫）

植物幼胚の磨砕液によるテトラゾリウム塩還元におけるコハク酸脱水素酵素の関与

佐 藤 七 郎*

Sitiro SATO*: Studies on the Participation of Succinic Dehydrogenase in the Reduction of Tetrazolium Salt by Plant Embryo Homogenates.**

この 実 験 の 目 的

この実験にはふたつの目的がある。その1はテトラゾリウム塩（2, 3, 5-triphenyltetrazolium chloride 以下 TTC と略記す）の還元によつてコハク酸脱水素酵素活性を検出できるかどうかを磨砕液（Homogenate）でたしかめること。その2は青酸の阻害作用が磨砕液でもみとめられるかどうかをたしかめることである。

著者[14, 15]はツルナシインゲンの発芽前の幼胚軸・幼根の生切片で，TTC 還元反応によつてコハク酸脱水素酵素活性を検出することが可能であることを立証したが，なお他の材料について，それが不可能であるという報告[3,4,6,7,22]もすくなくないので，前回と同一の材料について，切片におけるよりもより直接的な証明の可能な磨砕液でこのことをたしかめる必要が生じた。他方，切片を用いる実験では青酸が阻害作用をあらわし，その原因が不明であつた。この阻害作用が酵素あるいは酵素系じたいの性質に由来するものであるか，それともより高次の細胞構造たとえば原形質膜の透過性にたいする作用のごときものに由来するものであるか，をたしかめる必要が生じたのである。

材 料 と 実 験 方 法

試料　ツルナシインゲン *Phaseolus vulgaris*（マスターピース品種）を用う。乾燥したタネを25°C で水浸，17〜20 時間後に胚軸と幼根をはず

す。大部分が胚軸で幼根の部分はごく小さい。この水浸時間では細胞はじゆうぶんに水をふくんでいるが，まだ種皮をやぶつて発根する状態まではいたつていない。1回の実験に約200 コのタネから幼根つき胚軸（以下便宜上幼胚と記す）をとり，これに 0.25 M ショ糖をふくむ 0.1 M リン酸緩衝液（pH 8.0，あらかじめ冷却しておく）を約 14〜16ml 加えて冷却しながら磨砕。4枚重ねのガーゼでこしてから遠心分離（2000 × g）10 分によつてでんぷん粒，細胞膜などをのぞき上清を磨砕液として試料にする。

反応　反応はツンベルグ管内でおこなわせた。ツンベルグ管の各室の内容は：

主　管
（全液量 3 ml）
$\begin{cases} 0.25 M \text{ ショ糖をふくむ } 0.1 M \\ \text{リン酸緩衝液 pH 8.0 ……… 2 ml} \\ \text{磨砕液*** ………………………… 1} \end{cases}$

副　管
（全液量 2 ml）
$\begin{cases} 0.1\% \text{ TTC 水溶液 ……… 0.5} \\ 0.1 M \text{コハク酸ソーダ水溶液 0.5} \\ \text{蒸溜水 …………………… 1.0} \end{cases}$

薬物の作用をしらべるときは蒸溜水の代りに薬物溶液を入れる。管内の空気を窒素ガスにいれかえ37°Cで5分間あたためてから両管の内容をまぜて反応を開始させる。一定時間後に冷却して反応をとめ形成されたフォルマザン（triphenylformazan）を定量する。

定量　上記の混液を 4 ml とり，氷サク酸 10 ml と，トルエン 7 ml の混合液に加えて放置す

* 東京大学理学部植物学教室細胞学研究室（Botanical Institute, Faculty of Science, University of Tokyo）

** Contributions from the Divisions of Genetics and of Cytology, Botanical Institute, Faculty of Science, University of Tokyo, No.361.

*** 磨砕液 1 ml は 15〜20mg の乾燥タンパクをふくむ。

ると，上清約 5 ml にフォルマザンが移る。これを
日立製作所製光電比色計（フィルター No. 47，波
長 455～475 mμ.）で比色定量する。フォルマザン
の量は光学密度［log (I₀/I)］であらわす。反応
のつよさを比較するときには反応速度の相対値
（対照を 100 とする）R であらわした。

実 験 結 果

はじめにこの実験方法じたいにたいする検討な
ど 2～3 の予備実験をおこない，つぎにコハク酸
脱水素酵素の阻害剤の作用をしらべ，さいごに青
酸の作用について実験する。

**(1) 磨砕液中のフォルマザン量と抽出物の光
学密度との関係** 上記の主管と副管の内容を混合
した完全反応液（緩衝液，磨砕液*，TTC，コハ
ク酸ソーダ，蒸溜水：計 5 ml）にハイドロ亜硫
酸ソーダの結晶を加えて TTC を完全に還元し，
これを磨砕液でうすめて各濃度のフォルマザン液
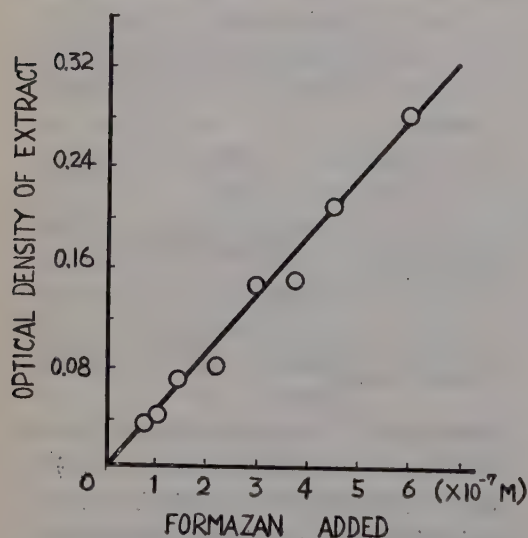をつくり，ついで酵素の反応をおこなわせること
なく，ただちに抽出して光学密度をはかる。その

Fig. 1. Relation between formazan concentra-
tion in reaction mixture and optical density of
formazan solution extracted therefrom; No
enzymatic reaction. Formazan was extracted
with 17 ml. acetic toluene (glacial acetic acid,
10 ml.; toluene, 7 ml.) from the mixture (4 ml.
each) which contained known amounts of for-
mazan and the optical density was determined
spectrophotometrically.

結果は Fig. 1 に示すとおりで，フォルマザンの
サク酸トルエン溶液は Beer の法則にしたがい濃
度と光学密度とは比例関係にある。したがつて，
この範囲では光学密度を測ることによつてフォル
マザン量すなわち還元された TTC の量をみつも
ることができる。

(2) コハク酸の濃度 各管中に与えるコハク
酸塩の濃度をかえて反応を行わせ，60 分後の還
元量を比較すると Tab. 1 のようになる。反応速
度（相対値）R は 0.01 M のコハク酸塩を加え
たばあいを 100 とした値であらわした。コハク

Table 1. Effect of substrate concentration
on the rate of TTC reduction by bean embryo
extract. Extraction: About 200 ungerminated
embryos were homogenized in an ice-chilled
mortar with about 15 ml. of cold phosphate
buffer (0.1 M, pH 8.0) containing 0.25 M
sucrose. The homogenates were filtered
through gauze and centrifuged lightly. The
resulting supernate (cell free extract) was
employed as the enzyme preparation. Reac-
tion mixture: Tissue extract, 1 ml. (about
20 mg. protein); phosphate buffer (0.1 M, pH
8.0, containing 0.25 M sucrose), 2ml.; sodium
succinate(0-0.02 M final concentration), 5 ml.;
distilled water, 1.0 ml. Total volume: 5ml.
The substrate and TTC were tipped from the
side arm into the main tube after temperature
equilibration for 5 min. Thunberg tubes; gas
phase, 100% nitrogen, 37°C; reaction time 60
min. The formazan produced was determined
as described above (see the explanation for
Fig. 1).

Concentration of Na-succinate (M)	0	0.001	0.01	0.02
Formazan produc-ed (optical density)	0.047	0.075	0.139	0.097
Reaction rate (R)	34	54	100	70

R: Relative reaction rate as % of that with
0.01 M substrate.

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* このばあいにかぎり発芽後のやや時期のすすんだ幼植物の磨砕液を用いた。

酸は明瞭な促進作用をもち*，この実験の限りでは 0.01 M のときに最大の還元量を与えた。よって他の実験ではいずれもこの濃度のコハク酸ソーダを基質として加えることにした。

　（3）　**磨砕液の濃度**　前記の方法で作つた磨砕液を磨砕用液（ショ糖をふくむ緩衝液）でうすめて種々の濃度の磨砕液をつくり，同一条件で反応させて，1 時間後の還元量をはかると Fig. 2 の結果がえられた。還元速度 R は磨砕液の濃度がひくいときにはきわめてよわいが，濃度があるていど以上高いときには，磨砕液濃度と直線的関係を示す。

　（4）　**還元の時間的経過**　同一条件で反応を開始させてから一定の時間間隔で 2 時間にわたつて還元量の増加を測つた（Fig. 3）。反応はすくなくも 2 時間のあいだは同一の速度で進行することがみられる。他の一つの実験例では反応は 4.5 時間にわたつてほぼ直線的に進行した。

　（5）　**反応液中の滲透価**　本実験の反応中ショ糖の濃度は 0.20 M で細胞液とほぼ等張であるが，この濃度を 0.05, 0.65, 0.95 M にかえたばあいの還元量を Fig. 4 に比較す。図から，還元のつよさは反応液中の滲透価のえいきようをうけ，優張液中でつよく，劣張液中でよわいことがわかる。さいきん，高等植物でもコハク酸脱水素酵素がミトコンドリヤなどの大型細胞質顆粒にふくまれていることが明かになり[13]，著者の実験材料でもミトコンドリヤをふくむ細胞においてコハク酸脱水素酵素の活性がみとめられたから[17,18]，本実験における TTC の還元には大型細胞質顆粒の中の酵素も関与していると考えなければならない。したがつて Fig. 4 にあらわれた滲透価のえいきようが細胞質顆粒の含水状態にたいするそれに起因するものとも想像される。しかし他方，ネズミの肝ぞう，心ぞう，腎ぞうの細胞質顆粒を破解して遊離状態にしたコハク酸酸化酵素系も滲透
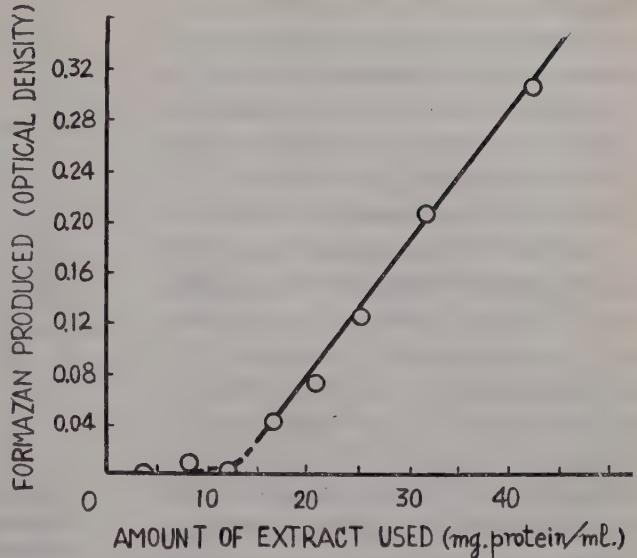

Fig. 2. Reaction rate as a function of the amount of extract. Substrate, 0.01 M ; tissue extract, 4 mg. ~42 mg. protein/ml. Otherwise as described above (see Table 1).
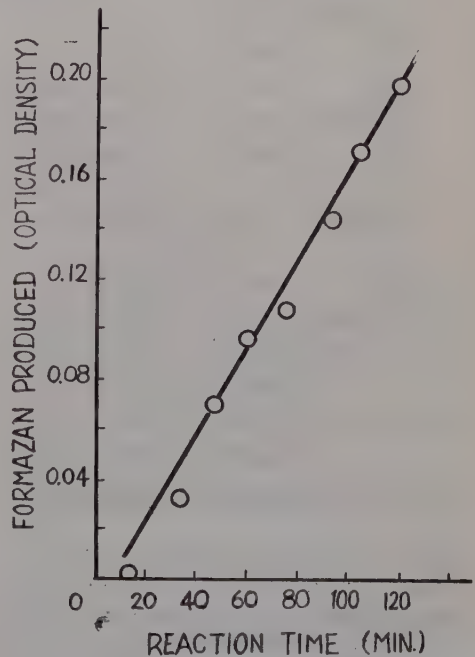

Fig. 3. Time course of TTC reduction. Substrate, sodium succinate 0.01 M.

────────────────

* コハク酸の促進作用はメチレン青を指示薬としたときにもみとめられた。

価の直接のえいきようをうけると報告されている
し，つぎの実験で示されるように，内源的 (endo-
genous) な基質による還元にたいするえいきよう
は基質添加のばあいのそれとことなる様相をてい
したので，これらのデーターだけからいずれとも
断定することはできない。

Fig. 4 に示した結果は冒頭に記した処方によつ
て 0.01 M のコハク酸塩を基質として与えたとき
のものである。コハク酸塩を加えなくても若干の
還元がおこることは Tab. 1 にみられるとおりで
ある。この内源的な基質による還元の部分にたい
する滲透価の作用は Fig. 5 に示す。このばあい
には極端な優張液でないかぎり滲透価のえいきよ
うはみとめがたい。したがつて Fig. 4 に示され
た滲透価のえいきようはコハク酸の関与する反応
系の部分に由来するものとおもわれる。極端に高
い滲透圧のもと（1 M 以上）では還元は減退し
た。

Fig. 5. Rate of endogenous reduction of
TTC (without the addition of succinate).
In striking contrast to the results with the
addition of the substrate (Fig. 4) the rate
was not appreciably affected by the hyper-
tonicity of reaction medium with higher
sucrose concentrations (1 M), however,
more or less marked depression of the
reaction rate was noticed. The experi-
ments were carrid out as stated above
(see Fig. 4), but without the addition of
the substrate.

Fig. 4. Dependence of the rate of TTC
reduction as affected by the tonicity of
reaction medium. Experiments were car-
ried out as stated above, (see Table 1),
but with 0.01 M sodium succinate and
with varied concentrations of sucrose.

(6) ショ糖が基質となるか この実験では胚
を磨砕するときに滲透的に作用するていどの高濃
度のショ糖液を用いなければ，磨砕液の還元活性
が不安定であり，かつ低いので，それを用いてい
るが，そうしたばあい磨砕液にショ糖が入ること
になり，ショ糖が TTC 還元の基質として使われ
ているかもしれないという可能性も考えられる。
このことをたしかめるために，ショ糖をふくまな
い緩衝液 (0.1 M) で調整した磨砕に 0～0.8 M
のショ糖を加えた*ときの還元を比較すると Fig.
6 のようになつた。図は加えたショ糖の濃度が 0
のときを 100 とした比較値を示す。このばあいは
0.4 M ていどでやや阻害的な傾向があらわれ，そ
れより低い濃度でもほとんど促進的な作用はみら
れない。Fig. 4. の結果を Fig. 5, 6 のそれと比
較すれば前者のばあいにおけるショ糖の促進的効
果はショ糖を基質とする TTC 還元反応にたいす
るものでないことは明かである。

* ショ糖は最終濃度が図示のとおりになるように主管の緩衝液中に加えておいた。
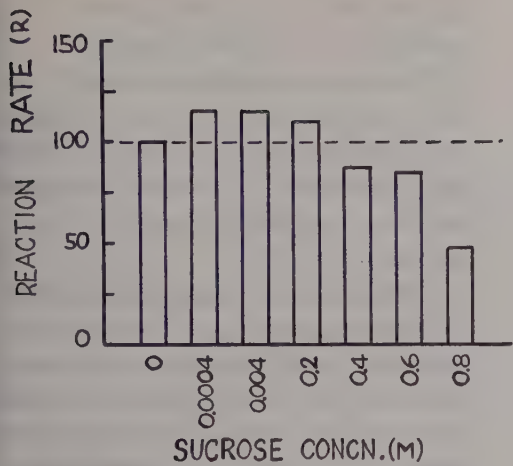
Fig. 6. Effect of various sucrose concentrations on reaction rate. The homogenate was prepared with hypotonic phosphate buffer (0.1 M) without the addition of sucrose. The reduction of TTC was not markedly enhanced by the addition of sucrose 0.0004 M to 0.2 M; but a certain deterioration with 0.4 M to 0.8 M sucrose (final concentrations).

(7)　磨砕液の酵素活性の安定さ　反応開始前の温度平衡のための加温は5分間としたが，これを65分間に延長したときの還元は，対照（5分）；R＝100と比較するとR＝90となる。これはこのていど（37℃，60分）の条件では磨砕液の酵素活性はあまり低下せず，比較的安定であることを示す。(4)にのべたようにTTC還元の時間的経過は37℃においても2時間（Fig. 3のばあい）あるいはそれ以上ながい間直線的にすすむ。このことは基質の有無がこの酵素系の安定性にいちじるしいえいきようを与えることに原因するように思われる。

(8)　熱失活　切片では52℃，30分間の加熱処理によつて反応が完全に不活性化された。本実験でも磨砕液をあらかじめ52℃で30分間前処理すると完全に失活して基質を与えてもTTCを還元しなくなる。これはこの条件における還元には熱にかなり不安定な酵素が関与していることをものがたる。

(9)　各種阻害剤の作用　上のようにして測られたTTC還元にコハク酸脱水素酵素が関与して

いるかどうかを検証するためにそれらにたいするマロン酸，オキサロサク酸，ピロ燐酸，赤血塩，亜ヒ酸，モノヨードサク酸の作用をしらべた。これらはコハク酸脱水素酵素の特異的あるいは非特異的阻害剤としてしられているものである。[2,11,19,23] このうちオキサロサク酸と赤血塩いがいはすでに切片を用いた実験で，いずれも阻害作用のあることがたしかめられている。[14,15] 本実験はいずれも基質として0.01Mのコハク酸塩の存在する条件の下で行つた。その結果をTab. 2.に示す。

Table 2. Effects of various inhibitors on TTC reduction. Experiments were carried out as stated above (see Table 1), but with 0.01 M substrate and with the addition of the inhibitor solution instead of distilled water in the control.

Inhibitor	Concentration (M)	Reaction rate (R)
None (control)	——	(100)
Malonate	0.001	94
〃	0.01	28
〃	0.02	0
Oxaloacetate	0.001	0
Pyrophosphate	0.001	7
Ferricyanide	0.001	19
Arsenite	0.001	76
Monoiodoacetate	0.01	74

マロン酸は基質として同時に与えたコハク酸（0.01M）より低濃度（0.001M）では阻害が明かとはいいがたいが，等濃度またはそれ以上ではきわめてつよく反応を阻害する。

オキサロサク酸，およびピロ燐酸による阻害は低濃度でもきわめていちじるしい。

SH阻害剤の赤血塩も明かな阻害作用をあらわす。

亜ヒ酸はより軽度ではあるが，やはり阻害的である。

モノヨードサク酸はやや高い濃度では阻害作用をあらわす。それより低い濃度（0.001M）では作用があらわれないか，あるいは逆に促進作用をあらわす実験例があつた。*　モノヨードサク酸は

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* ただしこの促進作用がいかなる機作によつておこるかはまだ明かでない。

SH 基を有する多くの脱水素酵素にたいして阻害作用を示すものであるが, [2,11,19] それらのうちコハク酸脱水素酵素にたいしては他の脱水素酵素のばあいにくらべてやや高い濃度ではじめて阻害作用をあらわすことが動物組織の酵素についてしられている[2]が, 本実験の結果はこれとよく符合する。

以上の結果は本実験における TTC の還元にコハク酸脱水素酵素が関与するという結論と調和するものである。

(10)　**青酸カリおよび窒化ソーダのえいきよう**　青酸の作用を検討するに当つて, ある点でそれと近似の作用をもつところの窒化ソーダのえいきようをも同時に検討することは, 青酸の作用の機作を推察する手がかりを与えるものとかんがえ, 青酸カリおよび窒化ソーダをそれぞれ前実験とおなじようにして添加した。その結果は Tab. 3 にしめす。

Table 3.　Effects of KCN and NaN$_3$ on TTC reduction rate.　Experiments were carried out as stated above (see Table 2).

Inhibitor	Concentration (M)	Reaction rate (R)
None (control)	——	(100)
KCN	0.0001	85
〃	0.001	64
〃	0.1	83
NaN$_3$	0.001	99
〃	0.01	73

青酸カリは 0.001 M ていどのうすい濃度ですでに明瞭な阻害作用をしめした。この結果は切片を用いた実験のばあいと一致する。したがつて切片のばあいの青酸阻害が, 主として透過性にたいする作用に由来するとはいえない。もつと直接的な作用をかんがえなければならない。他方窒化ソーダは 0.01M でやや阻害的にはたらくが, 0.001 M では作用をみとめることができない。したがつて, TTC 還元の過程において青酸によつて阻害される段階は窒化ソーダによつてはほとんど作用をうけない性質のものである。

磨砕液における青酸の阻害作用はメチレン青を指示薬としたときもみとめられた。

なお磨砕液のばあいには切片のばあいとことな

り, 有酸素条件（空気中）では反応はほとんど進行しない。

(11)　**その他 2～3 の薬物のえいきよう**　この酵素反応については諸薬物のえいきようの特異性がしばしば問題とされている[12]ので, いくつかの関連ある薬物の作用をしらべてみた。それらの結果はいずれも本論文の結論と調和するものであつた。(Tab. 4)。

サク酸は切片の実験では緩衝液として通常使用される濃度で阻害作用のあることがみとめられたのであるが, 磨砕液ではもつと低濃度で阻害をあらわした。ただしこのばあい阻害はあまりつよいものではなく 0.001 M ではあらわれない。コハク酸とともに TCA サイクル上の基質であるクエン酸およびそれに近い位置にあるグルタミン酸はコハク酸ほどではないが促進作用をしめした。発酵阻害剤のフッ化ソーダと低濃度のモノヨードサク酸はかえつて促進的であつた。

Table 4.　Effects of various reagents on TTC reduction rate.　Experiments were carried out as stated above (see Table 2), except that sucrose, acetate, citrate, and glutamate were added in place of succinate.

Reagent	Concentration (M)	Reaction rate (R)
None (control)	——	(100)
Sucrose*	0.004	114
Acetate	0.001	101
〃	0.01	74
Citrate	0.001	117
Glutamate	0.001	148
Na-fluoride	0.01	117
Monoiodoacetate	0.001	131
〃	0.01	74

* See the explanation for Fig. 6.

考　　察

コハク酸脱水素酵素の関与について　切片によるテトラゾリウム塩 (TTC) の還元にコハク酸脱水素酵素が関与するという報告はすくなくない。[19,29] しかしこれを磨砕液で追証した研究はすくない。Kun and Abood[8] は動物組織の磨砕液でこの酵素の活性を TTC 還元反応で定量することが可能であるといつているが, Fred and Knight

(4) はペニシリウムのばあいについて，TTC 還元はコハク酸脱水素酵素の特異的な作用に対応するものでないといい，Bodine, Lu and West[3] は動物組織の磨砕液をつかつて TTC 還元で特定の酵素を検出することはできないといつている。植物組織では Jensen, Sacks and Baldauski[6] がトウモロコシ胚のアセトン標品でコハク酸添加の効果を否定し，ついで Smith[22] は同じ材料を低温下で磨砕した磨砕液でくわしい検討をくわえてこれに賛成し，TTC でコハク酸脱水素酵素活性を測定することは危険であると結論した。その後 Anderson[1] はイエバエの筋肉の磨砕液でコハク酸脱水素酵素の関与をたしかめたが，コハク酸脱水素酵素の関与をみとめた研究はいずれも動物組織を用いており，植物組織の磨砕液を用いた上記 2 報告はいずれもその関与を否定している。Glock and Jensen[5] はカラスムギの磨砕液でコハク酸脱水素酵素活性を定量したが，このばあいには，TTC の 2 分子縮合体 p p'–diphenylenebis-2-(3. 5 diphenyltetrazolium chloride) を用いている。

　本実験では基質，阻害剤の作用がコハク酸脱水素酵素の特性をよく反映し，TTC の還元にこの脱水素酵素がなんらかの形で関与していることはたがいないものとおもわれる。TTC への直接の H 供与体がなんであるかは本研究の結果からは結論できないが，あるばあいについては H 伝達体としてフラボプロテインの関与が証明されている（酵母，細菌）。[8 21 22]* Jensen et al. とのくいちがいは，かれらのばあいアセトン標品が用いられているので，標品調整のさいにコハク酸脱水素酵素が不活性化されていたのによると推察される。植物のコハク酸脱水素酵素は動物のばあいに比していちじるしく不安定であるといわれているからである。[13] 動物組織の磨砕液が用いられたさいにかぎつて肯定的な結果がえられているのもそのためであろう。Smith[22] はこの点に配慮して，低温下で磨砕した磨砕液を用いて実験をおこなつているがやはり否定的結果をえた。かれは磨砕のさいに劣張緩衝液（0.02 M）を用いているし，材料の相異もあるのでこの点については追試をこころみたい。上にのべたように本研究の結果から，すくなくともツルナシインゲンの発芽前の幼胚の磨砕液ではコハク酸の存在下における TTC 還元作用はコハク酸脱水素酵素の活性を反映するものであると結論することができる。

　なお酸化還元電位の比較から理論的に，TTC（pH 7.0 における $E'_0 = -0.083$ V）の還元はコハク酸脱水素酵素系（おなじく $E'_0 = 0.00$V）によつてはおこりえないとする主張[6] があるが，これは，TTC→Formazan の反応が不可逆であり後者が水に不溶性であることを考慮にいれたばあい正当とはいえない。

青酸の阻害作用について　TTC 還元反応にたいする青酸の阻害作用については，先の切片での実験 [14, 15] では酵素への直接的な作用のほかに細胞の透過性，原形質の膠質状態に関連する間接的な作用も考えられるので，事情はふくざつであるが，本研究において青酸が磨砕液においても明瞭な反応阻害をきたすことが示されたので，阻害が透過性にたいする作用によるという可能性は否定することができる。磨砕液でコハク酸脱水素酵素の関与をみとめた研究のうち Kun & Abood[8] は青酸はえいきようを与えないといい，Anderson[1] はその阻害作用をみとめた。後者によればこの阻害はチトクローム c の添加によつて減退するという。また著者の材料では胚の発育がすすむと，同じ条件で調整した磨砕液を用いても，青酸の作用が阻害から促進に転ずるという事実がある[16]。阻害の機構は TTC 還元反応系の構成と関連して論ぜられなければならない問題で，粗酵素を用いた実験からはあまり立いつたことはいえないので，ここでは追及しないこととする。

あ　と　が　き

　本研究のために終始有益なご意見をいただいた和田教授をはじめとする研究室の諸氏ならびに，本稿を閲読のうえ詳細にわたつて助言くださつた高宮教授，小倉助教授にあつく感謝の意を表します。

Summary

1. The reduction of TTC in the presence of embryo homogenates of *Phaseolus*

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* コハク酸脱水素酵素そのものがメタロフラボプロテインであとという報告もある。[9]

vulgaris was investigated by the colorimetrical determination of the amounts of formazan formed by the reaction under varied experimental conditions, and the results were compared with those obtained with the tissue slices (cf. previous papers).

2. The rate of TTC reduction was shown to be dependent on the presence of succinic acid in the reaction medium, and it was concluded that this reaction was due to the activity of succinic dehydrogenase in the homogenate.

3. The rate of TTC reduction was fairly constant for considerable reaction period, and the activity of the succinic dehydrogenase system was conveniently measured by determining the initial rate of formazan production.

4. In harmony with the above conjecture, the poisons known to inhibit the action of succinic dehydrogenase (malonate, oxaloacetate, pyrophosphate, ferricyanide, arsenite, monoiodoacetate) all inhibited the TTC reduction in the presence of sodium succinate.

5. The inhibitory action of potassium cyanide on the TTC reduction was demonstrated with homogenates as well as with slices of bean embryo. Sodium azide had no apparent effect on the reduction.

6. The seemingly contradictory results of other investigators concerning the reduction of TTC and especially the problem of participation of the succinic dehydrogenase on the process were discussed.

References

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Type を異にするトウモロコシ種子の多糖類生成
に関する酵素学的研究[*] III

Non-waxy トウモロコシの Q-酵素[**]

田　中　国　治[***]

Kuniji TANAKA[***]:　Enzymatic Studies on the Mechanism of Polysaccharide Formation in
Maize-Seed III.　Q-Enzyme of Non-Waxy Seeds

　著者は前報[11] で，Non-waxy トウモロコシ
(Country Gentleman × Wisconsin No. 690) の
種子の Q 酵素をジャガイモのアミロースに作用
させて 得た アミロペクチンの β-アミラーゼによ
る分解限が，ジャガイモの Q 酵素を作用させて
得たものよりも高く，またヨウ素呈色が青紫色で
あることなどから，トウモロコシの Q 酵素によ
って 得た アミロペクチン分枝のグルコース鎖長
は，ジャガイモの Q 酵素によって 得たものより
も長いものと考えた。

　今回はこの両酵素によってそれぞれ，分枝され
るグルコース連鎖の長さの比較をするために，ア
ミロース型の低分子のデキストリンを用いる方法
をとった。

　即ち，ジャガイモのQ-酵素によっては分枝さ
れるが，トウモロコシの酵素によっては分枝され
ないような，ある鎖長の直鎖デキストリンをまず
得ようとした。ジャガイモのQ酵素によって分枝
されるグルコース鎖長の下限は 40±10 である[6]
ので，ほぼその附近の鎖長のデキストリンを基質
に選び，トウモロコシの酵素による分枝作用をジ
ャガイモのQ-酵素の作用と比較した。Q 酵素に
よる分枝形成の有無は，反応生成物のβ-アミラー

ゼによる分解限が，デキストリン自体のものより
も低下するかどうかによってきめた。

　糖質トウモロコシの胚乳中には，多量のフィト
グリコーゲン[*] が含まれるが，その形成機構はま
だほとんど解っていない。こゝではこの物質が，
同じく糖質トウモロコシ胚乳中に形成されるグリ
コアミロース[**]から，Q-酵素の作用によって生成
するかどうかを検した。フィトグリコーゲンのβ-
アミラーゼによる分解限は 47%[4] であるから，分
解限が約 47% の反応生成物が生ずることを期待
してグリコアミロースにトウモロコシの Q-酵素
を作用させた。このばあい，グリコアミロースの
分枝の増大を促すために，アミロースを同時に添
加することも行った。

　それらの結果について報告する。

実　験　の　部

　酵素調製—Non-waxy (Country Gentleman ×
Wisconsin No. 690) 種子の Q-酵素は，前回[11]と
同じ方法で調製し，10 gm. の膨潤種子より得た
酵素標品を 7 ml. の水に溶解した。ジャガイモの
Q-酵素は Barker 等[1] の方法により調製し，100
ml. のジャガイモ搾汁より得た酵素標品を 20 ml.
の水に溶解した。ダイズ β-アミラーゼは Bourne

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*/, **/ 糖質トウモロコシ種子に存する水溶性多糖類で，両者の主な相違は，分枝程度において，フィトグ
　　　リコーゲンはグリコアミロースに対して著しく高い点にある[3]。こゝに用いた名称は，Sumner &
　　　Somers[9] の命名によった。

等[2] の方法により調製し，その 2.5 % 水溶液を 2 日間水道水に対して透析して用いた。

基質調製——アミロースはジャガイモでんぷんから調製した。前回[11] のには，なお多少 β-アミラーゼで分解されない部分があったが，これを除いたために，温水抽出液をチモールで処理する前に，3 G No. 4 のグラスフィルターで濾過した。本標品を 5% 硫酸で 100°C に 3 時間加水分解し，グルコースとして 93% 相当の還元力を認めた。また β-アミラーゼによって完全に加水分解された。

直鎖デキストリン約 1.1% のアミロース溶液 400 ml. に 5% H_2SO_4 100 ml. を加え，100°C に約 1 時間加熱後，水酸化バリウムによる硫酸除去，CO_2 通気によるバリウムの除去，濃縮，アルコール分別沈澱（45%〜60%），透析，アルコール再分別（45%〜60%）等の操作を順次行い，アルコール及びエーテルで洗い，P_2O_5 上で減圧乾燥して調製した。本標品は β-アミラーゼによって完全に加水分解され，Swanson等[10] の方法で測定した平均グルコース鎖長は 37 であった。

グリコアミロースは糖質トウモロコシの 1 種 Country Gentleman の種子から，Morris 等[5] の方法に準じて調製した。その水溶液は乳白色で，ヨウ素呈色は弱青色，純度は約 90% であった。

酵素作用の測定——デキストリンあるいはグリコアミロースに対する Q-酵素の作用はその強さを知るために，つねにアミロースに対する作用と比較した。反応混合液の組成は 0.3% 多糖類溶液 4.0 ml.（多糖類 0.15 gm. を数滴のエタノールで潤おし，0.5 N NaOH 10 ml. を加えて煮沸水浴上で溶解し，0.5 N H_2SO_4 でフェノールフタレーン中性とした後全量を 50 ml. とした），0.1 M クエン酸塩緩衝液（pH 6.7）2.0 ml. 及び Q-酵素液 1.0 ml. 反応温度 30°C。一定時間作用させた後，100°C に 10 分間加熱して Q 酵素を失活させ，冷却後 β-アミラーゼ溶液 1.0 ml. を添加して反応生成物を加水分解（30°C で 8〜24 時間）し，消化液 1.0 ml. をとって生じた麦芽糖を定量した（Somogyi の試薬 No. 60）[8]。生成麦芽糖%はこの β-アミラーゼ消化液 1.0 ml. 中に麦芽糖 1.5 mg. に相当する多糖類を含むものとして次の式から求めた。

$$生成麦芽糖\% = \frac{β\text{-アミラーゼ作用で生}成した麦芽糖\ mg.}{1.5\ mg.} \times 100$$

但し，グリコアミロースあるいはグリコアミロースとアミロースの混合物を基質として用いたばあいには，次の方法で基質溶液中の多糖類の濃度を求めた後，その β-アミラーゼ消化液 1.0 ml. 中に存する多糖類の麦芽糖相当量を得て生成麦芽糖%を求めた。即ち基質溶液 4.0 ml. に 7% H_2SO_4 10 ml. を加え，100°C に 3 時間還流冷却器を附して加水分解し，1 N NaOH でフェノールフタレーン中性とした後全量を 100 ml. とし，その 5.0 ml. をとって Shaffer-Somogyi の試薬 No. 50[7] で定量してグルコース量を得，それに 0.9 を乗じて，原液量に換算した。Q 酵素反応中，混在するアミラーゼによって分解される基質の量は僅少（麦芽糖として 5% 以下）であったので考慮しなかった。

グリコアミロースの β-アミラーゼによる分解限の測定条件は次のようにした。反応液組成：0.3% グリコアミロース（溶解の方法は先のばあいと同じ）4.0 ml.，0.1 M クエン酸塩緩衝液 2.0 ml.，水 1.0 ml. 及び β-アミラーゼ溶液 1.0 ml. 反応温度 30°C。反応液 1.0 ml. をとって生成した麦芽糖を定量し，酸加水分解法によって求めた多糖類濃度から生成麦芽糖%を算出した。

直鎖デキストリンに対するジャガイモ及びトウモロコシ Q-酵素の作用——デキストリンにそれぞれジャガイモ及びトウモロコシの Q-酵素を作用させたときの結果を Table I に示す。

ジャガイモの Q-酵素をデキストリンに作用させると，その生成物は明らかに β-アミラーゼによる分解限の低下（6%）を示したが，トウモロコシ Q-酵素を作用させたばあいにはこの低下が見られなかった。対照に用いたアミロースに対する両酵素の作用から解るように，この結果は両酵素標品の活性度の相違によるものではない。即ち，こゝに調製したデキストリンは，ジャガイモの Q-酵素によっては分枝されるが，トウモロコシの Q-酵素によって分枝されない境界附近にあることを示している。

フィトグリコーゲンの形成機構に関する実験——糖質トウモロコシの胚乳中に含まれるフィトグリコーゲンが，共存するグリコアミロースから，Q-酵素の作用によって生成するかどうかを検した。即ち，グリコアミロースにそのまゝあるいは，アミロースを混在させて Q-酵素を作用させ，分枝作用

Table I. Action of Q-enzyme from maize-seeds and potato tuber on an amylose-type dextrin. Reaction mixture contained 4.0 ml. of 0.3% polysaccharide, 2.0 ml. of 0.1 M citrate buffer (pH 6.7) and 1.0 ml. of enzyme solution. Reaction temperature 30°C. After incubation periods indicated Q-enzyme action was stopped by heating and the reaction product was further subjected to the action of β-amylase. With an aliquot of the digestion mixture maltose was estimated.

Source of Q-enzyme	Substrate	Incubation period with		Maltose produced by the action of β-amylase	
		Q-enzyme min.	β-amylase hr.	mg.	(average) %
Potato tuber	Amylose	0	14	1.36	
			16	1.36	91
		90	14	1.16	
			16	1.19	78
	Dextrin	0	14	1.36	
			16	1.36	91
		90	14	1.28	
			16	1.28	85
Maize-seed	Amylose	0	19	1.36	
			22	1.33	90
		130	19	0.94	
			22	0.97	64
	Dextrin	0	19	1.33	
			22	1.33	89
		130	19	1.36	
			22	1.33	90

の結果フィトグリコーゲンのβ-アミラーゼによる分解限（47%）に近似の値を示すような反応生成物が得られるかどうかを検した。このためにはまずグリコアミロース自体の β-アミラーゼによる分解限を求めた。

Fig. 1 に示すように，この分解限は約 57% である。

グリコアミロースに対する Q-酵素の作用：結果を Table II に示す。

対照のアミロースに対する分枝作用がほぼ終末（β-アミラーゼによる分解限 65%）近くまで（62%）進行するくらいに，酵素を十分に作用させたが，グリコアミロースからの反応生成物はグリコアミロース自体と同程度の分解限を示し，Q-酵素による効果はなにも見られなかつた。

アミロース存在下のグリコアミロースに対する Q-酵素の作用： 多糖類の全濃度は上の実験と同じにし，グリコアミロース：アミロースを 7：3 にした。この混合基質の β-アミラーゼによる分
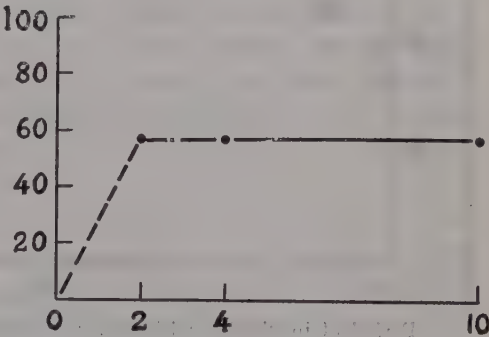
Fig. 1. Limit of hydrolysis by β-amylase of glycoamylose.
Reaction mixture contained 4.0 ml. of 0.3% glycoamylose, 2.0 ml. of 0.1M citrate buffer (pH 6.7), 1.0 ml. of water and 1.0 ml. of β-amylase solution. Incubation temperature, 30°C. With 1.0 ml. of aliquot, maltose was estimated. The concentration of glycoamylose in the substrate solution was determined by acid hydrolysis (5% sulfuric acid at 100°C for 3 hours) and subsequent estimation of glucose.

Table II.　Action of maize Q-enzyme on glycoamylose.
Experimental conditions were the same as indicated in Table I.

Substrate	Incubation period with		Maltose produced by the action of β-amylase	
	Q-enzyme hr.	β-amylase hr.	mg.	(average) %
Amylose	0	15	1.45	
		19	1.45	97
	6	15	0.97	
		19	0.97	65
Glycoamylose	0	15	0.71	
		19	0.74	60
	6	15	0.71	
		19	0.71	59

解限は，グリコアミロース及びアミロースの分解限がそれぞれ 57% 及び 100% であるから約 70% となるべき筈である。この基質にトウモロコシ Q-酵素を作用させた結果を Fig. 2 に示す。

結果は，基質の β-アミラーゼによる分解限は 69% で，終末反応生成物の分解限は約 59% である。この分解限 59% は，混じたアミロースがそのままアミロペクチン（分解限62%）[11]の形成だけに用いられ，グリコアミロースがそのまま残ったばあいの理論値（59%）に相当した。
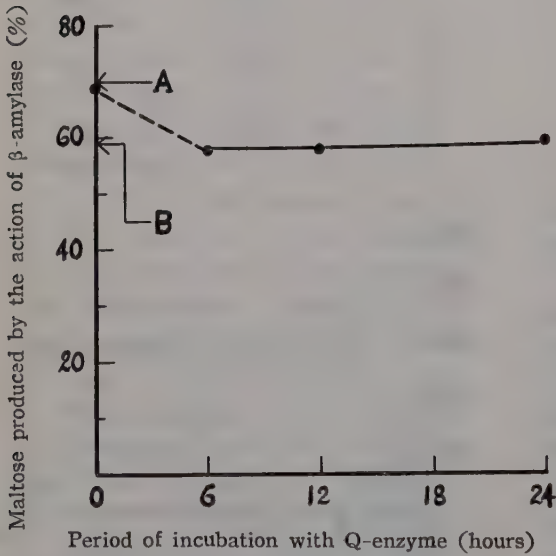
Fig. 2. Action of maize Q-enzyme on glycoamylose in the presence of potato amylose.
Substrate contained 7 parts of glycoamylose and 3 parts of potato amylose. (A) indicates the theoretical limit of β-amylolysis of the substrate. (B) indicates the theoretical limit of β-amylolysis of the reaction end product when amylose is converted into amylopectin and glycoamylose remains intact by the action of maize Q-enzyme.

考　察
本実験で用いた直鎖デキストリンは，ジャガイモの Q-酵素によって分枝されるが，トウモロコシの Q-酵素によっては分枝されない境界附近の鎖長をもっていることを明らかにした。このことは，トウモロコシの Q-酵素によってアミロースから調製したアミロペクチンの β-アミラーゼによる分解限が，ジャガイモの Q-酵素によって得たアミロペクチンのそれよりも高いという前回の結果[11]と共に，トウモロコシ Q-酵素によって分枝されるグルコース連鎖の鎖長は，ジャガイモ Q-酵素によるよりも長いことを意味している。このことは，また，アミロペクチンの直鎖部分のグルコース鎖長がそれぞれの Q-酵素の相対特異性によって決定されることを示している。著者は前報[11]で糖質トウモロコシの胚乳に含まれるフィトグリコーゲンは，アミロペクチンからトウモロコシの Q-酵素の作用によって生成するものではないと考えた。今回は別の経路として，フィトグリコーゲンが同じく糖質トウモロコシの胚乳中に形成

されるグリコアミロースから Q-酵素 の作用によって導かれるものではないかという点を実験的に調べた。しかし，グリコアミロースに，単独に，あるいはアミロースを添加したものに本 Q-酵素を作用させても，β-アミラーゼによる分解限が 47% に近くフィトグリコーゲンの性質をもつた反応生成物は得られなかつた。フィトグリコーゲンはグリコアミロース分子が，Q-酵素の作用によって分枝程度を高めるというような簡単な機構だ

けによって生成するものではないと考えられる。

Summary

1.　The Q-enzyme from non-waxy maize-seeds (Country Gentleman × Wisconsin No. 690) has been found to produce an amylopectin with longer branching chain than that of amylopectin synthesized by the Q-enzyme of potato, corroborating the previous finding of the author.

2.　An attempt to obtain phytoglycogen by the action upon glycoamylose of the Q-enzyme of the maize-seeds has been found unsuccessful. It appears unlikely to regard glycoamylose as a direct precursor of phytoglycogen.

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ラ ン 科 植 物 の 染 色 体 研 究 I

ラ ン 科 植 物 数 種 の 染 色 体

大 野　林 二 郎*・橋 本　昭 巳*

Rinjirō ŌNO and Akimi HASHIMOTO: Chromosome Sudies in Orchidaceae I
Chromosome Numbers of Four Species in Orchidaceae

ラン科植物の染色体については筆者等の調べた限りでは，約 36 属 86 種が報告されているにすぎない。従って，未だ調べられていないものゝ数は相当に多い。

その原因はこの科植物の中には比較的入手困難な深山又は高山に自生するものも相当多いためと思われる。

本報では 3 属 4 種について花粉母細胞，花粉粒或は体細胞分裂などで観察した染色体について報告する。

研究材料及び研究方法

材料はエゾチドリ (*Platanthera metabifolia* F. Maekawa)，キソチドリ (*P. ophrydioides* Fr. Schm.)，クモキリソウ (*Liparis Kumokiri* F. Maekawa) 及びサイハイラン (*Cremastra variabilis* Nakai) の 4 種であるが，すべてこれらは 1954 年 5 月下旬から 7 月下旬にかけて採集したものである。採集場所はエゾチドリ，キソチドリ及びクモキリソウの 3 種はいずれも 大千軒岳にて採集したものであり，サイハイランのみは函館山にて採集したものである。但し大千軒岳は函館市より西南方約 90 粁ほど離れた所に位置する標高 1072 米の山である。而してこれらのもの之採集された所はいずれも樹陰の湿気にとんだ地帯であった。

観察にあたっては花粉母細胞及び花粉粒の場合はすべて醋酸カーミンなすりつけ法によって染色した。またサイハイランの根端細胞は，抱水クロラールにて約40分間前処理し，ナワシン氏液で24時間固定，パラフィン法にて 10μ の切片を作り，ハイデンハイン氏鉄明礬ヘマトキシリン染色法にて染色した。

観 察 結 果

A.　ツレサギソウ属 (*Platanthera* L. C. Rich.)

この属に属するものゝ染色体研究は極めて少く，僅かに Ōno (1954) がノビネチドリ (*P. decipiens* Lindl.) で n＝21 を報告しているのみである。このたびはこの属における下記の 2 種類の染色体を明らかにすることができた。

1.　エゾチドリ (*Platanthera metabifolia* F. Maekawa)

本種は北海道においても大千軒岳の如き比較的高地に自生している植物であり，この植物はたやすくは得られない。

花粉母細胞の第 1 成熟分裂中期で，明らかに 21 個の二価染色体が核板に配列するのが見られた (Fig. 1)。中期より後期にかけての染色体分離の行動を見るに，染色体はそれぞれ均等に両極に分れてゆき，何等異常は認められなかった。

中期における染色体の大きさ及び形態では，先ず大きさは皆ほゞ同大であり，それぞれの間ではそれほど大きな違いはない。

形態では楕円形又は中間にくぼみを持つやゝ長形の染色体が数個見られただけであった。

2.　キソチドリ (*P. ophrydioides* Fr. Schm.)

本種は比較的広範な地域に分布し，北海道，本州，四国，九州などの高山の針葉樹林中に分布する植物である。

このたびの材料は大千軒岳の山麓地附近で発見されたものである。

本種では花粉母細胞第 1 成熟分裂のみが観察せられた。成熟分裂の中期核板では 20 個の二価染色体が数えられた (Fig. 2)。染色体の形態は円形又は楕円形で，これらの間にはそれほど大きさの

* 北海道学芸大学函館分校生物学教室
　Biological Institute, Hakodate Branch, Hokkaido Gakugei University

差異は見られない。また分裂はすべて正常であった。

B. クモキリソウ属 (*Liparis* L. C. Rich.)

この属の染色体研究は Miduno (1940) が，*L. Loeselii* (n＝16, 2n＝32), *L. nervosa* (n＝21, 2n＝42) の 2 種について報告しているのみである。こゝでは更に 1 種について報告する。

3. クモキリソウ (*Liparis Kumokiri* F. Maekawa)

本種の分布も比較的広範な地域にわたり，北海道，本州，四国，九州にまで及んでいる。このたびの材料はすべて大千軒岳のものである。

花粉母細胞第 1 成熟分裂は時期が遅く残念乍ら見られなかった。花粉粒核分裂では明らかに n＝13 の染色体を観察することができた (Fig. 3)。この染色体では僅かばかりの核型的特徴が認められる。即ち先ず大きさにおいては，比較的他より大きい 5 個の染色体が観察される。而してこれらの 5 個の大形の染色体中，やゝ V 字形に近いものと，J 字形のものがそれぞれ 1 個ずつ見られ，他の 3 個は棒形又は楕円形のものである。残余の 8 個の比較的小さな 染色体 は楕円形又は円形である。

さらにこの分裂においては どの核板でも皆 13 個の染色体を有し，その数の少い特殊な花粉粒は見られなかった。

C. サイハイラン属 (*Cremastra* Lindl.)

この属の植物の染色体研究は筆者等の調べた限りでは，まだなされていないようである。筆者等はこゝに 1 種について最初に報告する。

4. サイハイラン (*Cremastra variabilis* Nakai)

この種の分布は広く，千島，北海道より南は九州に至る山地樹下に自生する植物である。このたびの材料は函館山にて採集した約 10 個体である。

観察は花粉母細胞及び根端における体細胞分裂の両者について行われた。先ず花粉母細胞では，割合に明瞭な像が少かつたが，第 1 成熟分裂中期で 24 個の二価染色体が数えられた (Fig.4)。これら 24 個の染色体の大きさはほゞ同大で，それぞれの間では余り違いは見られない。形態も円形又は楕円形で特異な形をしたものは見られなかった。

次に体細胞分裂中期核板では明らかに 2n＝48 を数えることができた (Fig. 5)。その大きさは花粉母細胞の場合と同様，その間には余り極端な差異はない。形態はすべて特徴的でなく，いずれ
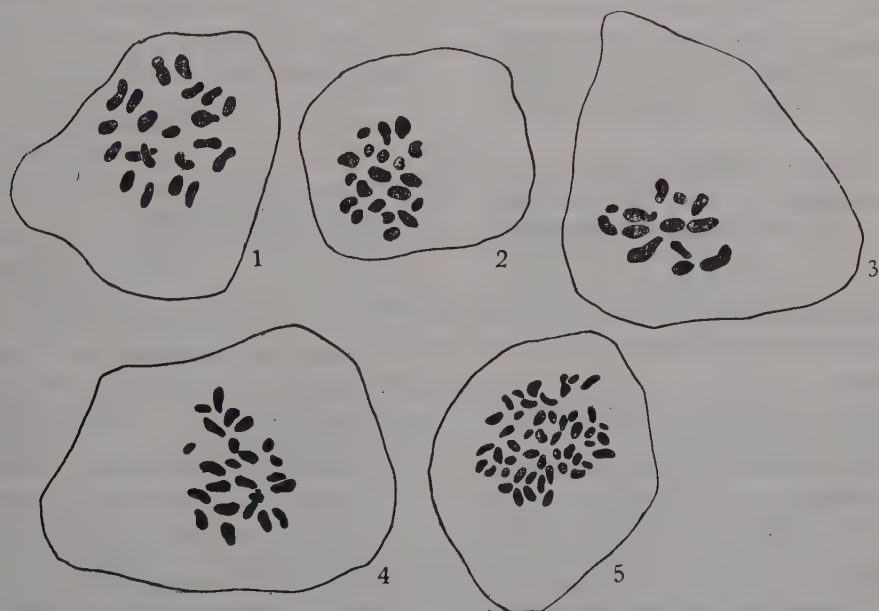
Figs. 1–5. 1–2. Meiotic chromosomes in PMCs, 1. *P. metabifolia* F. Maekawa. 2. *P. ophrydioides* Fr. Schm. 3. Haploid chromosome set in pollen grain division of *L. Kumokiri* F. Maekawa. 4. Meiotic chromosomes in *C. variabilis* Nakai. 5. Somatic chromosomes in RTC of *C. variabilis* Nakai. ×1700.

も円形, 楕円形又は棒形である。

　以上ラン科植物 4 種の染色体観察の結果では, 一般に染色体の大きさは小形であり, またその形

態においては余り特徴的なものはないようである。なお染色体数と系統については今後多くの種類を調べた上で論ずる積りである。

Summary

　1.　In the present study, chromosome numbers of 4 species in Orchidaceae were counted by the authors.

　2.　Chromosome numbers of *Platanthera metabifolia* F. Maekawa and *P. ophrydioides* Fr. Schm. in meiosis were found as n=21 and n=20, respectively. And that of *Liparis Kumokiri* F. Maekawa in the pollen nuclear division was n=13, and for *Cremastra variabilis* Nakai it was n=24 in the PMC and 2n=48 in the root tip cells.

　3.　Karyologically, the chromosomes of 4 species here reported are all of nearly equal form and size.

　4.　The behaviour of chromosomes in meiosis was regular.

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本　会　記　事

支　部　通　信

関東支部

　5 月例会 (5 月 26 日, 於東大理, 植物講義室) 佐藤七郎: 組織化学の TTC 法のための基礎的実験. 水島正美: 下北半島中心部泥炭地の植物相.

近畿支部

　例会 (5 月 13 日, 於南龍蔵寺) 永井　進: メタセコイヤ等の組織によるテトラゾリウム塩の還元に就いて. 高田英夫, 平岡純一: 酵母食塩抵抗菌のメチレンブルー吸収と陰イオン共存との関係. 藤田安二: ヒカゲヒメジソに就いて. 河原　明: スギ科植物の乾燥と吸收との関係. 北村四郎: カラコルムヒンズークシの植物に就いて.

中国四国支部

　支部大会 (5 月 12～14 日, 於山口大文理学部, 日本動物学会中国四国支部と共催)
公開講演. 下斗米直昌: 遺伝と染色体. 川村智治郎: 動物の雌雄性.
植物学会講演. 高木恭介: 水稲の生育に於ける銅の影響に就て. 塩見隆行: ヤマノイモ属植物の根の褐変現象について. 生駒義博, 西山一穂*, 池沢知一: 砂丘植物の解剖学的研究 (第二報). 生駒義博, 川本満喜夫*: 鳥取県の羊歯植物の分布について. 生駒義博: 境港水道は植物分布上一線を劃す. 森　千乔: 山口県東部の島での暖地性植物の分布. 日出武敏: 東部四国の新しいチリモ

の 2, 3 について. 安藤久次: ニスビキカヤゴケ *Parella vernicosa* Lindb. 3 亜種の分布と生態について. 鈴木兵二: 日本産オオミヅゴケの変異堀川芳雄: 日本の高山帯. 笠原安夫: 耕起の時期回数と圃場雑草群落. 越智春美: 汎太平洋地域およびインドマレー地域に於ける *Bryum ramosum* (Hook.) Mitt. とその近縁種 2, 3 について. 越智春美, 佐々木俊夫*: ジャゴケの細胞液滲透価について. 福田八十楠: 砂丘植物の蒸散について. 賀来章輔: 長日植物の光週反応に及ぼす NAA の影響. 斎藤雄一: 成長調節物質の撒布がクロマツの花性の分化並びに花の発育に及ぼす影響. 中原清士*, 畑中千歳: ヒガンバナの Saccharase 及び Transfructosidase に就いて. 米山　穣: マツ―アベマキ群落内の酵母菌について. 猪野俊平, 西林長朗*: ミツイシコンブの遊走子嚢発生と遊走子形成. 木村劼二: 不和合性複相菌糸による帽菌類の diploidisation について. 田草川春重: ヒルムシロ属植物の倍数性と含水量との関係. 神野太郎: キイチゴ属 2, 3 の倍数体. 栗田正秀: イチリンソウ属およびこれと近縁な 2 属における核型比較. 内川　勇: 普通コムギにおける第 IX 染色体の不分離現象について. 田中隆荘: ハマカンギクの人為合成の研究. 金子賢一郎: キク属に於けるゲノム倍加と花粉管伸長との関係について. 下斗米直昌, 益森静生: 瀬戸内海西部沼岸島嶼産ノデギクの形態学的, 細胞学的研究. 下斗米直昌, 杉山　一: ミヤマヨメナの種内倍数性.

Flowering Responses in *Pharbitis Nil* as Influenced by the Removal of Photoperiodically Induced Leaves

by Shun-ichiro IMAMURA* and Atsushi TAKIMOTO*

今村駿一郎* ・ 滝 本 　敦*：　日長感応を受けた葉の除去がアサガォの花芽形成に及ぼす影響

Many investigations have indicated that relatively long dark periods rather than relatively short photoperiod are a factor essential to the initiation of flower primordia in short day plants under natural conditions. In a leaf kept in darkness some change or changes may take place bringing about photoperiodic induction. This "status" is transmitted from the leaves to the growing point where morphogenetic processes occur resulting in floral initiation. In two-branched plants of *Xanthium* the receptor branch cannot produce flower primordia when the donor branch is removed after receiving two short days, whereas after 3 short days floral initiation occurs on the receptor despite of the removal of the donor branch (5). The case is also the same in leaves. *Xanthium* and *Pharbitis*, which initiate flower primorida by receiving a single dark period, develop flowers when the induced leaves are removed before any sign of flower formation is detectable at the growing point (6, 14). Recently the defoliation technique was used for investigating the sensitivity of leaves of different age and transmission of the stimulus from the leaf (8,9,10,13, 14, 15). From 1951 to 1952 research was carried out on the influence of the removal of photoinduced leaves upon flowering responses in *Pharbitis Nil*. The main results were summarily reported previously (6), and a detailed description is the subject of the present paper.

I. Material and methods. Three varieties of *Pharbitis Nil.*, " Violet ", " Scarlet "** and " Tendan ", were used in the present research. Strain " Scarlet " is very similar to " Violet ", which was used in the previous experiments (6,7), not only in external characteristics but also in photoperiodic behavior. The most apparent difference is the position of the first two foliage leaves on the main axis, being in " Scarlet " in close proximity. " Tendan " is believed to be a wild variety from North-China and its critical dark period seems to be somewhat shorter than in the other two varieties.

In a wooden box 20 or 25 plants were planted in 4 or 5 rows and at the start of the experiment the inferior individuals were removed. To eliminate the error

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  * Laboratory of Applied Botany, Faculty of Agriculture, Kyoto University. 京都大学農学部
    応用植物学研究室
** We owe this strain to the kindness of the late Dr. Tanemori Megata.

due to the individuality of boxes, experiments were designed in such a way that the rows of plants receiving different treatments were growing side by side in one box in randomized order.

They were grown since the seeds had been sown in the greenhouse under continuous illumination supplemented with incandescent filament lamps at night. At varying times after the dark treatment of each given duration, the donor leaf was removed and the flower initiation in the receptor bud was examined after about 2 weeks.

In most cases about one-month-old seedlings were defoliated and debudded to a single just expanded young leaf and its axillary bud, which were used as donor* and receptor*, respectively. In some experiments two leaves were left intact as donors.

II. Influence of removal of the donor leaf at the end of dark treatment of various duration. Four boxes with 20 plants in each were transferred to the dark room from a continuously illuminated bench. After single dark treatments of 12, 16, 18 and 24 hours' duration each box was returned to light and the donor leaf, which was the only leaf on the plant left intact, was removed at the top of the petiole. The plants were then grown under continuous illumination until harvested.

In one experiment, shown in Table 1 (Exp. 1), the lots which received a 12 or 16 hour dark period before the removal of the leaf blade did not produce a single flower primordium. In the lot of 18 hour dark period one plant out of 20 initiated a flower primordium. In the last lot which received a 24 hour dark period 8 plants

Table 1. Influence of the removal of the induced leaf upon
flower initiation in " Scarlet ".

| | Responses | Duration of dark period before removal of leaf (hours) | | | |
		12	16	18	24
Exp. 1*	No. of plants with flowers / No. of plants observed	0/20	0/20	1/20	8/20
	No. of flowers	0	0	1	14
Exp. 2**	No. of plants with flowers / No. of plants observed	0/20	0/14	1/17	7/16
	No. of flowers	0	0	6	44

* Sown on April 21, experiment started on May 28, 1952.
** Sown on April 23, experiment started on June 5, 1952.

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* The terms " donor leaf " and receptor bud " have been usually used when both were located on different branches of one individual or on branches of two graft partners. In the present and following papers the same terms will be applied also when those organs will be located on one and the same branch.

initiated 14 flower primordia in total.　Another experiment showed quite similar results (Exp. 2).　From these experiments we may conclude that the stimulus transmitted from the donor leaf within 16 hours is not strong enough to cause floral initiation in its axillary bud.

III. Response upon defoliation in relation to the number of leaves subjeted to dark treatment.　The time, in which the effective amounts of stimulus are transmitted from donor leaf, may be affected by internal and external conditions.　Among environmental factors temperature may have the most striking effect.　As to the internal factors the number and age of the donor leaves may be the most important factors.　Further experiments were carried out to investigate the effect of the number of leaves subjected to darkness.　Plants with the first or second leaf and plants with both first and second leaves were subjected to dark treatments of different duration, at the end of which the leaf blade or blades were entirely removed at the top of the petiole.　The axillary bud of the second leaf served as receptor bud.　In the experiment represented in Table 2 the plants which had only the first leaf left intact did not produce flower primordia upon defoliation after dark treatment lasting up to 19 hours, whereas 4 out of 23 plants produced flower primordia when defoliated after darkness of 20 hours.　Four out of 21 plants with the second leaf initiated flowers

Table 2.　Response upon the removal of induced leaves.　Axillary bud of the 2nd node served as receptor bud.　"Scarlet"

(Sown on April 23, experiment started on June 9, 1952)

Duration of dark period (hours)	Response	Leaves subjected to dark period		
		1st leaf	2nd leaf	1st and 2nd leaf
17	No. of plants with flowers / No. of plants observed	0/23	0/23	2/24
	No. of flowers	0	0	2
18	No. of plants with flowers / No. of plants observed	0/33	0/34	4/34
	No. of flowers	0	0	5
19	No. of plants with flowers / No. of plants observed	0/23	4/21	7/23
	No. of flowers	0	5	11
20	No. of plants with flowers / No. of plants observed	4/23	6/21	16/23
	No. of flowers	5	9	26

when the donor was removed after a dark period of 19 hours' duration. In all lots with both the first and second leaves some plants initiated flower primodia and the number of the flowering plants as well as that of the flower primordia increased with the increasing duration of the dark period. The time required for transmission of the stimulus from the donor leaf was less than 17 hours in plants with two leaves, in those with the second leaf between 18 and 19 hours and in those with the first leaf between 19 and 20 hours. Thus an additive effect of stimuli transmitted from two leaves was apparent.

In another similar experiment shown in Table 3, no plants which had only the first leaf as donor produced flower primordia, even if they were subjected to darkness of 22 hours' duration before having been defoliated. Some of the plants in all lots with the second leaf initiated flower primordia, and increasing response with increasing duration of the dark period was also obvious. The flowering response in plants with two leaves was more evident than in the foregoing lot. The induction by dark treatment given to the first leaf, which is not enough of itself to cause flowering, added up to the stimulus originated from the second leaf and the flowering response was intensified.

That the time of transmission in plants with the first leaf is longer than in

Table 3.　Response upon the removal of induced leaves.　Axillary bud
of the second node served as receptor. " Violet "

(Sown on May 20, experiment started on June 13, 1952)

Duration of dark period before defoliation (hours)	Response	Leaves receiving dark period.		
		1st leaf	2nd leaf	1st and 2nd leaf
16	No. of plants with flowers / No. of plants observed	0/19	2/19	3/18
	No. of flowers	0	2	6
18	No. of plants with flowers / No. of plants observed	0/20	3/19	14/18
	No. of flowers	0	9	32
20	No. of plants with flowers / No. of plants observed	0/19	11/19	17/20
	No. of flowers	0	33	68
22	No. of plants with flowers / No. of plants observed	0/20	17/20	18/20
	No. of flowers	0	62	71

plants with the second leaf, may be attributable to the following facts. In the first place, the first leaf is inferior in sensitivity to the younger second one just expanded (3, 7). In the second place, the distance between donor and receptor is longer in plants with the first leaf than in those with the second leaf. We cannot conclude with certainty which of the factors is the principal cause of the difference in response. There are some indications that the stimulus decreases on its way while passing through the stem, which will be reported on another occasion.

IV. Stability of photoperiodic induction in the illuminated leaf. Further experiments were performed with the intention to investigate the influence of light upon a leaf which has received dark treatment in excess of the critical duration.

Eight boxes with 20 plants in each were placed in the dark room. After 16 hours they were all returned to the illuminated bench. The donor leaves of one lot were cut off immediately after the transfer from the dark room. The remaining lots were defoliated various hours after the dark treatment as indicated in Table 4. The lot defoliated immediately after the dark period remained vegetative. In the lot which received a 16 hour dark and thereafter a 2 hour light period 4 plants out of 19 produced 5 flower primordia in total. With increasing duration of the light period following the dark period not only the number of flowering plants but also that of flower primordia was increased. The increase was remarkable in the first few hours of the light period and became later less conspicuous. After a 24 hour photoperiod the plants initiated as many flower primordia as the plants, whose donor leaf was left intact until harvested. The experiment shows clearly that a leaf, when once induced, can supply the floral stimulus to the receptor bud even if it is illuminated.

Table 4. Stability of photoperiodic induction in light; leaves given 16 hour dark period were removed after having been exposed to light periods of different duration. "Violet"

(Sown on June 29, experiment started on July 18, 1951)

Responses	Duration of light period given before removal of the leaf which was previously subjected to dark period of 16 hours' duration (hours)							
	0	2	4	6	8	12	24	not removed
No. of plants with flowers / No. of plants observed	0/20	4/19	10/20	9/20	11/20	17/20	17/20	19/20
No. of flowers	0	5	20	19	21	27	40	48

In Table 5 the results of another similar experiment with the variety "Tendan" are represented. The responses of this variety are very similar to those of the foregoing experiment.

Table 5.　Stability of photoperiodic induction in light; leaves given 14 and 16
hour dark periods were removed after having been exposed to light
periods of different duration. "Tendan"

(Sown on April 3, experiment started on May 2, 1952)

Response	Photoperiodic treatment given prior to the removal of the donor leaf (indicated by abbreviations)								
	16^hd $+0^h$l	16^hd $+2^h$l	16^hd $+4^h$l	16^hd $+\infty^h$l	14^hd $+0^h$l	14^hd $+2^h$l	14^hd $+4^h$l	14^hd $+8^h$l	14^hd $+\infty^h$l
No. of plants with flowers / No. of plants observed	0/20	3/19	7/19	16/18	0/20	0/20	3/18	10/19	12/19
No. of flowers	0	3	8	31	0	0	8	24	24

Abbreviations proposed by Takimoto for indicating photoperiodic cycles (16). For example 16^hd $+4^h$l indicates a dark period of 16 hours' duration followed by a light period of 4 hours.

In another experiment, the results of which are given in Table 6, 16 lots of 10 plants each were placed in the dark room and divided in four groups of 4 lots each. After 12 hours one group was returned to the continuously illuminated bench. After further 4, 6, 8 and 10 hours the respective lot was deprived of the donor leaf. The second group was removed to the illuminated bench, after having been kept 14 hours in the dark room. The defoliation of the four lots was made 2, 4, 6 and 8 hours after they were returned to the bench. The third group received a dark period of 16 hours' duration and was defoliated immediately, and 2, 4 and 6 hours after having been returned to light. The remaining 4 lots received 18 hours' dark period and were defoliated immediately, and 2, 4 and 6 hours after illumination. As shown in Table 6, among the plants which received a 12 hour dark period flowering occurred only in one, which was defoliated 22 hours after the beginning of dark treatment. In the lots of 14 hour dark period also one plant flowered. In the other lots which received longer dark periods, i.e. of 16 and 18 hours, the flowering responses became evident with the delay of defoliation. Increase in response with increasing delay of defoliation could be seen only in plants which received a dark period of sufficient duration. This was more clearly shown in the following experiment.

Five groups of boxes containing 20 plants each were subjected to dark treatment of 12, 14, 16, 18 and 20 hours' duration. Each of the first 4 groups consisted of 5 boxes, and the last group comprised 4 boxes. Four boxes, one from each of the first four groups, were defoliated 18 hours after the beginning of the dark treatment. Thus they received light periods of 6, 4, 2 and 0 hours' duration before defoliation. In 3 series of 5 boxes each, one box from each of all five groups were defoliated 20, 22 and 24 hours respectively after the beginning of dark treatment. The remaining 5 boxes from each group were not defoliated. The results are shown in Table 7. The group which received a dark period of 12 hours' duration initiated less flower primordia than the other groups, even when the donor leaf was not removed. In the

Table 6. Stability of the induction in light. Leaves given dark period were removed after exposure to light period of different duration. "Violet"

(Sown on July 7, experiment started on August 1, 1952)

Duration of the dark period (hours)	Response		Time of removal of the leaf from the start of dark treatment (hours)				
			16	18	20	22	24
12	No. of plants with flowers	No. of plants observed	0/10	0/10	0/10	1/10	—
	No. of flowers		0	0	0	1	—
14	No. of plants with flowers	No. of plants observed	0/9	0/8	1/10	0/10	—
	No. of flowers		0	0	1	0	—
16	No. of plants with flowers	No. of plants observed	0/10	2/10	4/10	6/10	—
	No. of flowers		0	2	10	13	—
18	No. of plants with flowers	No. of plants observed	—	0/10	2/10	8/10	7/10
	No. of flowers		—	0	3	11	9

groups which received 14, 16 and 18 hour dark periods, more flower primodia were initiated with the increasing delay of defoliation. The highest increment in response is found between the two lots which were defoliated after 18 and 20 hours. It is remarkable that the last group which received 20 hour dark period was far inferior in flowering response, and no increase in response with delayed defoliation was observed, a fact, which will be considered later in detail.

V. Discussion. From the results obtained it was revealed that the time in which the flower inducing stimulus is transported from the leaf in effective amounts to cause minimal flowering response in an axillary bud is about 14 to 16 hours. Even if the amount of the stimulus produced within a definite period in a single leaf is not sufficient, it will add up to the stimulus supplied by another leaf, whereby the response is intensified and flower initiation occurs, This evidence indicates that the amount of photoperiodic stimulus depends upon the number of leaves or, strictly speaking, upon the dimension of the leaf area subjected to dark treatment.

It is known that, in short day plants light inserted into a dark period decreases or annuls the effect of dark treatment. This is also the case with *Pharbitis Nil.* On the contrary, light given to a leaf after a long dark period does not destroy the induction. The illuminated leaf can continue to supply the stimulus to the growing point giving rise to a more pronounced flowering response. Whether, in this plant too, light given after the dark period has a stabilizing effect on induction, as reported

Table 7.　Stability of induction in light. Leaves given dark treatment were removed after exposure to light periods of different duration. "Violet"

(Sown on July 31, experiment started on August 28, 1952)

Duration of the dark period (hours)	Response	Time of removal of the leaf from the beginning of dark treatment (hours.)				
		18	20	22	24	not removed
12	No. of plants with flowers / No. of plants observed	1/19	3/20	1/18	1/15	3/20
	No. of flowers	1	4	1	1	3
14	No. of plants with flowers / No. of plants observed	3/19	10/18	11/20	9/17	12/20
	No. of flowers	3	18	20	18	22
16	No. of plants with flowers / No. of plants observed	6/19	16/20	15/19	17/19	14/17
	No. of flowers	7	30	29	33	33
18	No. of plants with flowers / No. of plants observed	2/20	13/18	15/19	17/19	15/18
	No. of flowers	2	31	34	48	52
20	No. of plants with flowers / No. of plants observed	—	4/20	3/18	3/19	4/19
	No. of flowers	—	8	8	9	13

recently by Lockhart and Hamner for *Xanthium* (11), cannot be decided without further experiments.

In some of the above experiments a long dark period was less effective than darkness of shorter duration in initiating flower primordia. This is clearly shown in Table 6, and in Table 5 in lesser degree a peculiar phenomenon, which must be considered in detail.

According to the theory of Bünning the characteristic responses of long- and short-day plants to day-length depend upon a rhythmical alternation of sensitivity to light occurring within the plants (1, 2). Two phases are postulated: photophile and scotophile. During the former, exposure to light promotes flowering; during the latter, light either inhibits or has no effect upon flowering. These alternating phases are of approximately 12 hours' duration. In the above mentioned experiment with a dark period of 20 hours the plants may have received darkness in the photophile phase of the next day, and thus the effect of dark treatment may have been reduced. In many short day plants it has been observed that prolonged darkness over a certain

duration is less effective in flower initiation. A dark period of 20 hours' duration may be beyond the optimum and may include the "descending phase" of the dark period in the sense of Gregory (4).

The above mentioned experiments were undertaken in summer in the greenhouse, where the temperature was not controlled and often rose to high degrees. As the plants were transferred to a dark room in the greenhouse from 5 p.m. until afternoon of the next day, it is highly probable that the temperature near the end of the long dark period may have been far beyond the optimum for photoperiodic induction and thus the responses might have been reduced. That an abnormally high temperature of short duration given in the dark period has a similar inhibitory effect as the light interruption was reported recently by Nakayama (12). We cannot decide without further experiments which alternative should be accepted, as we have no detailed information either on the diurnal rhythm of the physioloigcal activity or the "descending phase" of the dark period in the plant used in the present research.

Summary

  1) The effect of the removal of a photoinduced leaf upon flowering response in *Pharbitis Nil* was studied in plants which had only one leaf and the bud in its axil left. Upon the removal of the donor leaf after a 14–16 hour dark period a minimal flowering response occurred in the developing axillary bud. This indicates that in this duration the stimulus was transmitted from the leaf in a sufficient amount to effect flowering. With increasing dark period before defoliation the response increases.

  2) The effect of a dark period of sufficient duration is not destroyed by a following light period. Delayed defoliation results in increased response, even if the photoinduced leaf is illuminated.

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Das Wuchshormon des Fruchtkörpers von *Agaricus campestris* L.

(Vorläufige Mitteilung)

von Takashi URAYAMA*

浦山隆司*: ハラタケ（西洋マツタケ）子実体の生長ホルモン（予報）

Wenn ein junger Fruchtkörper (von ungefähr 2.5–3.5 cm Höhe) des *Agaricus campestris* L. in der Zentrallinie des Stiels der Länge nach durchgeschnitten wird, so krümmt sich die Wachstumspartie[1] des Stiels nach der Schnittfläche hin. Die Krümmung wird dabei nicht etwa durch Wasserverlust an der Schnittfläche hervorgerufen, da sich ein Fruchtkörper, dessen Schnittfläche man mit Lanolin oder feuchten Gasen behandelt, ebenso verhält.

Der Fruchtkörper setzt das Wachstum fort, auch wenn die obere Hälfte des Hutes mit Nadeln durchstochen oder sogar ganz abgeschnitten wird, soweit noch Lamellen vorhanden sind.

Schneidet man dagegen den Fruchtkörper in zwei parallelen, zur Zentrallinie des Stiels symmetrischen Ebenen durch (Abb. 1), so wächst der Stiel ganz gerade empor.
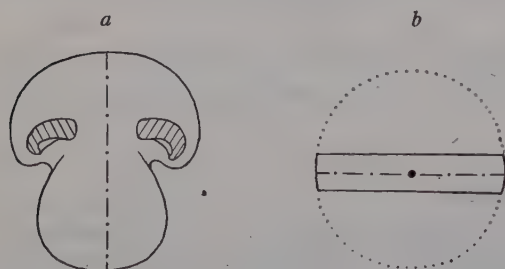

Abb.1. Paralleles Abschneiden des Fruchtkörpers.

a) Seitenansicht.
b) Oberflächenansicht.

(Lamellen schraffiert.)

Wenn man dann die Lamellen einseitig oder asymmetrisch entfernt oder durch Behandlung mit Trockeneis einseitig erfrieren lässt, so krümmt sich der Stiel immer nach der stärker beschädigten Seite hin (Abb. 2c). Es genügt, wenn die Lamellen in wenigen Punkten mit dem Fruchtkörper Verbindung haben, um auch das Wachstum des gegenüberliegenden Stielteils zu gewährleisten.

Dabei treten aber Entfaltung und Wachstum des Huts stärker an derjenigen

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* Laboratorium für Angewandte Botanik, Landwirtschaftliche Fakultät, Kyoto Unievrsität
  京都大学農学部応用植物学研究室

1) Die maximale Wachstumspartie des Stiels von *Agaricus campestris* liegt, wie bei anderen Pilzen, zwischen Ring und Hut.
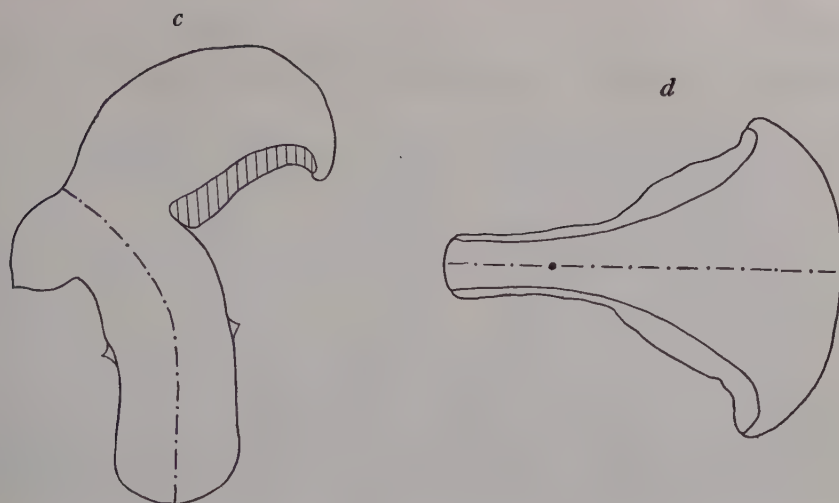
Abb. 2. Wachstum des Fruchtkörpers nach einseitigem Abschneiden des Hutes.

Seite auf, die grössere Lamellenteile besitzt (Abb. 2c, d).

Das Vorhandensein des Wuchsstoffs wurde sichergestellt durch Aufsetzen der abgeschnit tenen Lamellenpartie auf ein Agarblöckchen, welches auf die von den Lamellen befreite Schnittfläche geklebt wurde (Abb. 3).

Ein *Avena*-Test mit den aus den Lamellen in Agar-Agar diffundierten Substanzen fiel ebenfalls positiv aus. Die Anwendung von Wuchsstoffen höherer Pflanzen, wie Indolessigsäure (1:10³, 1:10⁵), α-Naphthalenessigsäure (1:10³, 1:10⁵), hingegen rief keine Krümmung hervor.

Auf Grund dieser Versuche kommt der Gedanke nahe, dass die Lamelle ein Wachstumsprinzip od. -prinzipien produziert, die von hier aus auf Stiel und Hut wachstumsreizend wirken.

Abb. 3. Diffusionsversuch des Wuchshormons durch Agarstückchen (punkiert). Links Agarstückchen ohne Lamelle.

Das Prinzip hat aber, wie es scheint, mit den auf *Avena*-Koleoptil wirksamen Substanzen, die gleichfalls von den Lamellen produziert werden oder zumindest in ihnen vorhanden sind, keine direkte Beziehung.

Diese Experimente wurden im Morimoto-Laboratorium für Künstliche Kultur Essbarer Pilze in Momoyama bei Kyoto gemacht.
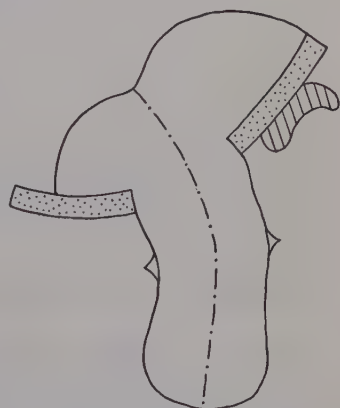
The Effect of Kinetin on Leaf Growth

by Susumu KURAISHI* and F. Shigeo OKUMURA**

倉石　晋*・奥村重雄**：　葉の生長に及ぼすカイネチンの影響

Introduction

Adenine has been regarded as a leaf growth hormone (Galston and Hand, 1949; J. Bonner and Galston, 1952), since D. M. Bonner and Haagen-Smit (1939) found that the leaf growth was accelerated by adenine and also by hypoxanthine which is one of the precursors of adenine. On the leaf of monocotyledone, however, de Ropp (1945) found the ineffectiveness of adenine. So it has generally been thought that adenine is a leaf growth factor for dicotyledone. As shown in previous paper (Kuraishi, 1956), adenine has an effect on the growth of *Raphanus* leaf only in very young stage. Recently, Miller et al. (1955 a, b) and Okumura (1955) found a remarkable cell division of tobacco wound callus tissue by the addition of IAA (indole acetic acid) and autoclaved DNA (desoxyribonucleic acid), though neither fresh nor dry weight increase could be clearly observed. From the autoclaved DNA, they succeded in taking out a kind of purine (6-furfurylaminopurine) that stimulates cell division and named it "Kinetin". In this paper the authors tried to ascertain whether kinetin stimulates the leaf growth as adenine or not.

Method and Material

Raphanus sativus L. was selected for this research as done by D. M. Bonner, Haagen-Smit and Went (1938). The variety "Riso Daikon" (Ideal Radish) was used. The seeds are sown in the flower pots in open air. When the first foliage leaves become about 0.6 cm² in surface area, the time after germination being about 10–20 days, the pots are transferred into the dark room and kept for about 24 hours. After that, the first foliage leaves are cut from the plants and punched out with a sharp cork borer, of which diameter is approximately 5 mm. The cut disks are carefully washed with distilled water two or three times, and shaken in distilled water for about five minutes to ensure the mixing of all the sections (ca. 1,000 disks). For three hours they are kept floating on distilled water in the dark room, then fifteen disks of them are, respectively, transferred to each Petri dish containing

* Botanical Institute, Faculty of Science, University of Tokyo. 東京大学理学部植物学教室
** Department of Chemistry, Faculty of Engineering, University of Tokushima. 徳島大学工学部応用化学教室

10 c.c. of the culture solution (Table 1). Before transferred to the dishes, disks are care-

Table 1.

The components of medium

Ca(NO₃)₂·4H₂O ....1.50 gr.
KCl ....0.25 gr.
MgSO₄·7H₂O ....0.25 gr.
KH₂PO₄ ....0.05 gr.
Sucrose ....20.0 gr.
Water...1000 c.c.

fully wiped off with filter papers, lest any drop of water on the upper surface disturbs their respiration (the radish leaf is amphistomatical). All these operations were done under the weak safety red light in the dark room and the close attention was paid to keep the disks safe. The Petri dishes are placed in an incubator at 30°C. After the incubation finished, they are wiped off by a filter paper within a few seconds and their fresh weights are determined to a milligram with a torsion balance. These procedures are done in such a uniform manner, that the error from varing degrees of wipe appears to be negligible. For the measurement of leaf area, they are placed between a pair of thin glass plates on the printing paper to obtain a shadow print, which is later cut out and weighed. After the fresh weight measurement they are brought in the oven at 70°C. Their dry weights are measured to a tenth of a milligram. The total nitrogen contents are determined by the micro-Kjeldahl method.

For the light incubation three Mazda fluorescent tubes "natural day light" were used.

On the day of experiment kinetin was dissolved which had been stored in the dry place, because the dissolved kinetin looks like to be decomposed within a week even in a refrigator at 4°C.

Results

It has been known for these thirty yeares (Priestly, 1925, 1926) that growth of the leaf is very sensitive to light. Then the experiments were performed, on one hand, to test whether the leaf growth with the addition of kinetin in the medium occurs, on the other hand, if it has an effect on leaf growth, whether its stimulating effect is accelerated with light. Kinetin was added to the medium at the concentration from 20,000 γ/l. to 5 γ/l. and the leaf disks were placed on their medium in Petri dishes, which were divided into two parts and incubated in dark and light.

The leaf disks were incubated for 20 hours at 30°C. And the experiments were repeated three times and at each of them duplicated lots of Petri dishes were used. All the results obtained were almost same. From Fig. 1, we can easily recognize the great stimulation to the leaf growth with kinetin both in light and dark, greater growth in light may be regarded as the light stimulation. In the concentration more than 10 γ/l. the acceleration of leaf growth is recognizable and the optimal growth is obtained between 500 γ/l. and 5,000 γ/l., being less effective more than 10,000 γ/l. This growth stimulation of leaf with kinetin under such low concentration resembles that of stem with auxin under its low concentration. We may thus conclude that
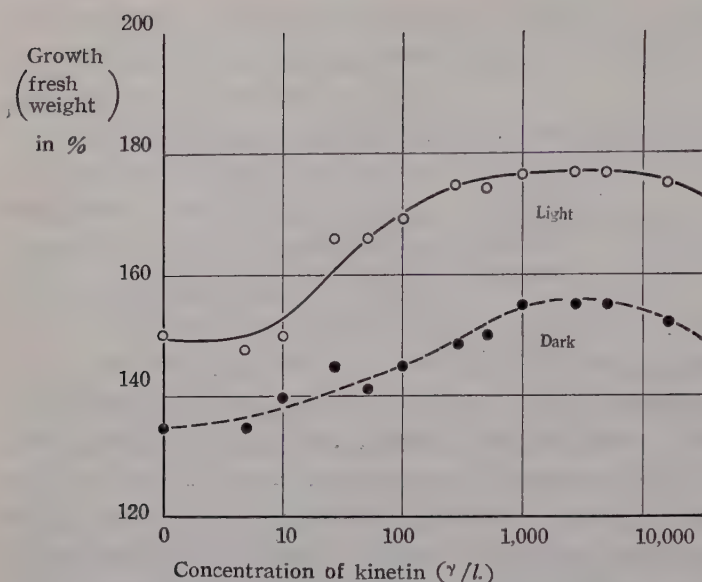
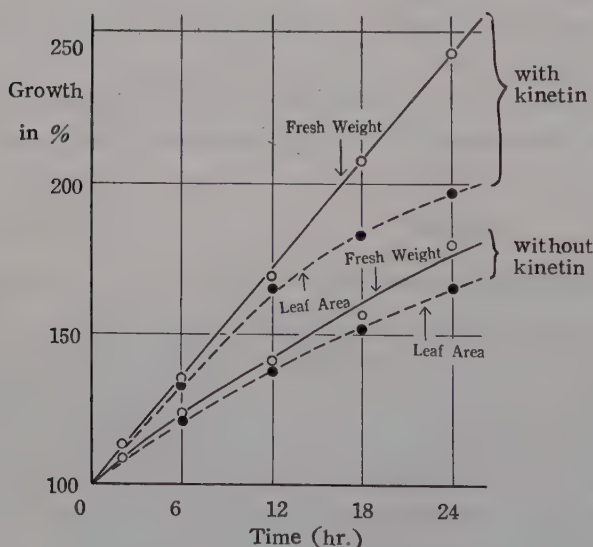Fig. 1   Leaf growth with kinetin under light and dark
          incubation.

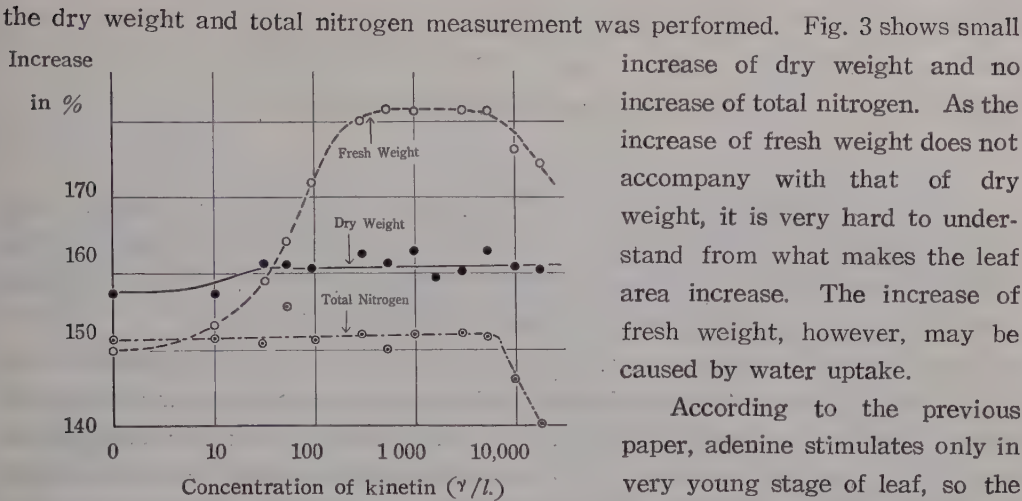Fig. 2   The time course of leaf growth

kinetin is a leaf growth factor or hormone, though the existence of kinetin in the plants is quite yet unknown.

The time course of leaf growth in the medium was next investigated. The media contain sucrose and are none sterilized, so, if long incubated, they fall into the contamination with bacteria. But it is not so remarkable within 20 hours. If kinetin is converted to one or some other substances either by bacteria or leaf itself adaptically, the time course of leaf growth will not progress linearly and will be a sigmoid form.

As shown in Fig. 2, the increase of their fresh weight show pretty proportionality with time even at first two hours after incubation. This may show direct utilization of kinetin on leaf growth, though its corrobora- tive proof could not be obtained for the sake of impossibility of measurement in shorter period after incubation. Fig. 2 also shows that the increases of the fresh weight and area of leaf disks during first 12 hours coincide with each other and in next 12 hours do not. Increasing the leaf area, leaf disks become convex and make it impossible to measure their exact area, so that was considered the cause of the divergence of the two curves.

As kinetin shows the remarkable increase in both fresh weight and leaf area,

the dry weight and total nitrogen measurement was performed. Fig. 3 shows small increase of dry weight and no increase of total nitrogen. As the increase of fresh weight does not accompany with that of dry weight, it is very hard to understand from what makes the leaf area increase. The increase of fresh weight, however, may be caused by water uptake.

Fig. 3　The increase of fresh weight, dry weight and total nitrogen with kinetin.

According to the previous paper, adenine stimulates only in very young stage of leaf, so the necessity occurs to test the effect of kinetin at different leaf stage. For this experiment three kinds of leaf, having different growth stage and different total area, i.e. 0.6, 0.9 and 6.0 cm². The leaf of 6.0 cm² was so old that it could not grow even in open air and 0.6 cm² showed the most vigorous growth both in outdoor and excised cultivation. They were incubated under 1000 lux for 20 hours at 30°C.

Table 2.　The effect of kinetin and adenine on different leaf stage. Figures are shown in relative to water control of fresh weights.

	Control	Adenine 10 mg./l.	Kinetin 1 mg./l.	Date
Leaf area 6.0 cm²	119.2	121.1	126.3	March　31th, 1956.
	121.2	120.0	130.1	May　　5th, 1956.
	115.0	116.1	121.5	May　　20th, 1956.
0.9 cm²	146.5	151.0	188.5	April　　7th, 1956.
	150.9	148.8	187.1	May　　1st, 1956.
	141.6	146.9	179.2	May　　10th, 1956.
0.6 cm²	162.6	174.0	217.5	April　　7th, 1956.
	215.6	218.0	270.5	April　19th, 1956.
	180.1	191.5	221.5	May　　5th, 1956.

1 mg./l. of kinetin and 10 mg./l. of adenine, the optimum concentration to leaf growth, were separately dissolved in the medium (table 1) and used for this investigation. As shown in table 2, adenine makes a little growth accelelation only at the smallest and not at the larger leaves. On the other hand, kinetin stimulates not only the growth of oldest leaf tested, but also far greater leaf growth than adenine does at any stage of leaf. It is very interesting that kinetin increases the leaf area which cannot grow more in the natural condition. Table 2 also shows some daily differences at each experiment and they are seemed by many kinds of different conditions

during the cultivation in open air.

Histological Observation

Miller et al. showed the increase of cell division by kinetin. According to table 2, it stimulates the matured leaf, which seems not to cause any more cell division. Then, in order to make sure whether it acts on the cell division as Miller mentioned, the histological studies had been performed using a leaf of 0.6 cm².

After 20 hours' incubation under the light condition at 30°C, the leaf disks were fixed with FAA (formalin acetic alcohol) under slight vacuum to allow quick penetration of the fixative. They were then dehydrated in alcohol and tertiary butyl alcohol and finally imbedded in a paraffin. The sections were cut transversely at a thickness of 10 μ and were stained with hematoxyline and fast green. It is of interest also that the increase in thickness with kinetin was rather uniform throughout the length of the sections. Though the number of cell layer was as same as control (the incubated leaf disks without kinetin), the increase in thickness was greater than the control. The leaf growth with kinetin may be raised with a general trend toward a greater degree of cell enlargement in sections and may be without a greater cell division (Fig. 4). And the leaves showed uniformely the increase of intercellular spaces, palisade parenchyma and spongy tissue, and seems not to increase only one tissue selectively. To confirm more clearly the phenomenon above mentioned, the histological studies, using a Sump method, were continued. The upper surfaces of leaves were replicated on small celluloid plates with a solution of Sump No. 2 and observed with microscope. Then, the vigorous cell division with kinetin could not be observed and only the enlargement of epidermis cells
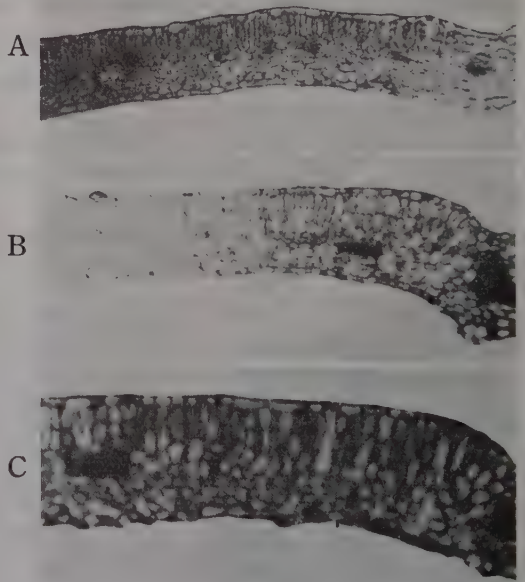

Fig. 4 The cross section of leaf. × 100.
A : Before incubation
B : Leaf section without kinetin
    after 20 hours' incubation.
C : Leaf section with kinetin after 20
    hours' incubation.

were found. The result by Sump method showed well agreement with that by microtome sections.

Addendum

It has been found to accelerate the leaf growth with kinetin, then the studies were continued to make sure whether kinetin acts in the same way as other growth hormone does.

Avena test : Being varied over a range from 1γ/l. to 20 mg./l. of kinetin, 2% of washed agar-agar blocks were soaked in these solutions for three hours and subjected to the Avena test with usual method. In these concentrations, any curvature could not be observed as the author reported on adenine.

Root test : For the root test, it is hard to obtain cress seeds and so *Brassica campestris* L. *var. parachinensis* Makino (Taisai) was used with an advice of Mr. H. Shibaoka. Kinetin was dissolved in distilled water, being varied over a range from 0.1γ/l. to 10 mg./l., and 2 c.c. of these solution was added into each Petri dish, 5.5 cm in diameter, in which was placed two sheets of filter paper, then, 10 Brassica seedlings (when their radicles became ca. 0.5 cm in length) grown in dark room were transferred into the dish and incubated for 20 hours. After incubation the length of root was measured (Fig. 4). The extreme growth inhibition was observed at the concentration of more than 10γ/l. and at any concentration the growth stimulation could not be found as auxin.
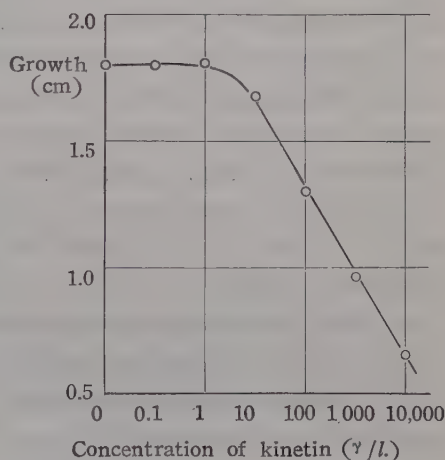

Fig. 4 Root test with kinetin.

Discussion

Miller et al. (1955 a) added kinetin and IAA to culture solution of tobacco wound callus and found the extreme increase of cell division, i.e. the ratio of cell numbers, treated/controls, was 31/1. Kinetin is a related compound to adenine that has been regarded as a leaf growth factor, so it may be no wonder that the former has an effect on leaf growth as the latter. It is hard to explain its growth accelerating mechanism by kinetin from the reason that kinetin is merely a related compound to adenine. The effect on leaf growth with the former may differ from that with the latter, considering effectiveness for leaf growth. In this experiment the ratio of cell numbers, treated/controls, was not 31/1 but smaller than 2/1. The leaf used in this experiment has adult cells and the tobacco wound callus has meristematic cells, so that both cell states are quite different. The materials may respond to kinetin in different manner at different cell states. It is very curious that one

substance stimulates cell enlargement, and not cell division in one experiment, while it has an effect on cell division, and not on cell enlargement in another experiment. One surmice is that kinetin may have two different actions. The second results different from the Miller's in this experiment is its activity showed without IAA. It is said that IAA has no effect on leaf growth (Avery, 1935). As IAA is made in the leaf, it may substitute the added IAA. If so, the leaf growth stimulation with kinetin maybe need not added IAA. But this problem needs more detailed study.

There are some great differences of action between auxin and kinetin i.e.kinetin neither increases root growth nor makes any curvatures of Avena coleoptile at tested conditions. Auxin, however, does not increase the leaf growth, the authors think that kinetin must not be included in a definition of auxin.

Summary

This study was intended to ascertain whether kinetin promotes a leaf growth by cell division or not, using the *Raphanus* leaf disks.

1. The leaves showed cell enlargement without cell division by the addition of kinetin, although it has been reported as a chemical which induces cell divisions in the case of tobacco wound callus.

2. It makes much increase of fresh weight and leaf area, and small of dry weight, but not total nitrogen. Its effectiveness was far greater than adenine on fresh weight and leaf area.

3. The effect of kinetin was found under both light and dark conditions.

4. Without the application of IAA kinetin acted on leaf growth.

5. It seemed to differ from the definition of auxin on the basis of the experiments of Avena curvature test, root test and leaf test.

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Physiological Studies on Growth and Morphogenesis of the Isolated Plant Cell Cultured *in vitro* II

The Growth and Morphogenesis of an Isolated Single Rhizoid Cell of Characeae*

by Tadashi SANDAN**

山段　忠： 遊離植物細胞の生長成形に関する生理学的研究 II
　　　　　車軸藻類の単一仮根細胞の生長成形

In the previous paper (Sandan 1955) the author reported that an isolated internodial cell of Characeae and even a cell fragment obtained from the internodial cell by strangulation were able to grow and develop new shoots and rhizoids when they were cultured *in vitro* in agar gel with suitable culture solutions.

The present report deals with an experiment performed using an isolated single rhizoid cell containing a single nucleus. It was shown that the potentiality for further growth and development is still maintained in such a cell.

Material and Methods

The rhizoid cell of *Nitella flexilis* which was developed from the internodial cell cultured *in vitro* was used as material in the present experiment. The internodial cell of *Nitella* was cultured according to the method described in the previous report (1955). Namely, the cell was kept in the vertical, normal position in the test tube which was filled by 4/5 with 0.8 % agar. The composition of the basic culture medium applied in the present work is as follows:

K_2HPO_4	0.02 g	$MgSO_4$	0.02 g
$FeSO_4$	0.005 g	d.w.	1 1

For the removal of fungi and bacteria from the agar gel 1 mg of Trichomycin P tablet (containing penicillin)*** and 0.5 mg of streptomycin respectively added to 50 cc of the culture medium.

The isolated internodial cell cultured in the test tube at room temperature under diffused light of about 30 lux develops several rhizoid cells from its basal end. These rhizoids reached 0.5 cm in length and 30 μ in width in about 20 days but they were still single cells by this time. Then the internodial cell was cut off from the rhizoid cells at its basal end with a very small scissors and the test tube containing the rhizoids was kept in a dark room for five or six days in order to remove the nodial

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cells which occasionally remained at the upper end of rhizoids after cutting off the internodial cell.　By this treatment these small nodial cells soon decayed accompanied with chlorosis and plasmolysis.　Then the rhizoid cells, which were generally connected with one another at their upper regions, were separated from one another with a sharp needle.

Thus several threads consisting of a single rhizoid cell were obtained in the agar gel.　These rhizoids were cultured in a dark room or under the illumination of 30 lux fluorescent light for ten hours a day.　We hereafter refer to the former case as the 'dark culture', and the latter case as the 'light culture' for simplicity's sake.　All experiments were carried out at the room temperature.

Results

1. Dark culture

The rhizoid cell continued to increase its size for several days after it was brought into the dark room.　The length and width of the rhizoid reached about 0.8 cm and 30μ respectively in 20 days.　Around this time the cell reproduction started to take place; several new cells which further increased their size were formed at the same time at the lower end of rhizoid.　The middle one among these new cells rapidly increased its size and became the first cell of the main 'root'. The other newly developed cells became the components of the branching 'root'. Later, these new cells reproduced themselves again at their lower end.　A similar process was repeated.　After 50 days' culture the length of the main root consisting of several rhizoid cells, which were newly formed, reached about 5 cm.　But after about 60 days' culture the decay of all rhizoid cells gradually occurred accompanying the decrease in the rate of protoplasmic streaming.

The nucleus in the young rhizoid cell was globular and comparatively large.　It generally moved around in the cell but it took its position at the root tip region when the cell reproduction occurred.　But in an old rhizoid the nucleus was deformed to a long plate-like shape.　At the time of cell reproduction it is likely that karyokinesis took place and the new daughter nuclei entered the cells which were newly formed.

2. Light culture

The increase in size and cell reproduction of the rhizoid in this case were the same as in the case of the dark culture conducted during 50 days.　In about 20 days, the length and width of the rhizoid reached 0.8 cm and 35μ respectively.　After 25 days the cell reproduction occurred rapidly at the basal end of the rhizoid.　In 50 days the whole length of the main root consisting of about five cells reached 6 cm.

The original rhizoid cell, which was single and was not fully developed at first, was transformed subsequently into the shape of a small internodial cell.　Many nuclei, which were probably formed from a single nucleus through mitosis, were recognized in the cell.　At the same time very small chloroplasts which gradually

increased their size appeared and were arranged in regular rows in the cell. Thus the white line, which is the zone having no chloroplasts, was also formed. After 70 days starch grains were observed in chloroplasts.

This changed cell was very similar to a normal internodial cell in appearance. After 80 days a new bud arose at the basal end of the internodial cell which was originally a rhizoid cell. This new bud became a small plant body consisting of small internodial cells, nodial cells and 'leaf' cells. Occasionally new small cells corresponding to nodial cells of the normal plant were formed from the lower end of the metamorphic cell and from these small cells new buds arose.

The differences between the normal internodial cell (abbr. NI) and the internodial cell which was formed from the rhizoid cell by metamorphosis (MI) can be summarized as follows: (1) The MI is very small in width. The width of the MI is about $50\,\mu$ and that of the NI is about $400\,\mu$. (2) The arrangement of chloroplasts in the MI is not so regular as that in the NI. (3) The white line
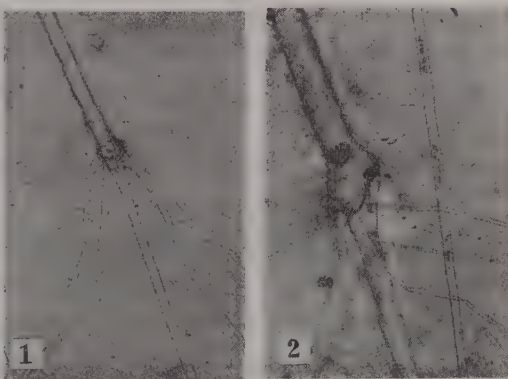

Fig. 1 A photograph showing the rhizoid cultured for 25 days. The rhizoid cell, which was cultured as a single cell at first, increased its size and reproduced several new cells at its basal end. ×80

Fig. 2 A photograph showing the rhizoid cultured for 55 days. The rhizoid cell, which was cultured as a single cell at first, is being metamorphosed into the shape of internodial cell. Small chloroplasts are observed. Nodial cells do not exist at its end. A normal rhizoid cell is seen at the right side of this changed cell. ×80

All rhizoids in these figures were cultured by the 'light culture'.

and the track of protoplasmic streaming in the MI are linear, while those in the NI are spiral. (4) The NI has small nodial cells at both its ends but generally there are no nodial cells in the MI.

After shooting, growth and cell reproduction were repeated in rhizoid cells and in new bud cells. Thus a single rhizoid cell, which was cultured *in vitro*, formed eventually a small complete plant consisting of 'leaf' cells, 'stem' cells and 'root' cells.

Discussion

Sasaki (1935), Kusunoki (1943) and Osterhout (1952) respectively reported that the isolated plant of Characeae occasionally formed rhizoids when the plant was kept in a medium such as the native pond-water in which the plant grew. But they did not try to culture an isolated rhizoid cell.

In the present experiment it was shown that a single rhizoid cell cultured in agar gel underwent growth and reproduction in the dark. Furthermore a fully developed rhizoid cell was metamorphosed into the shape of an internodial cell and

formed a new bud from its lower end. From these facts we may assume that the
agar gel has a favorable effect upon the growth and metamorphosis of the rhizoid
and further that a single rhizoid cell still has a potentiality of growth and cell
reproduction under both dark and light conditions. In the dark the rhizoid eventually
decayed after a long survival which involved both growth and cell reproduction.
But only weak light was sufficient for the rhizoid cell to metamorphose into the
shape of internodial cell and also to shoot a new bud. From these facts the mor-
phogenetic and reproductive abilities in the rhizoid seem to be activated in the pre-
sence of light.

When the metamorphosis of rhizoid occurred chloroplasts were formed accompanied
with karyokinesis and the formation of reserve materials in cytoplasm or in vacuole.
We may thus assume that a single rhizoid cell can form chloroplasts under weak
light and also that a new bud is formed with the aid of chloroplasts, nuclei and
reserve materials in the rhizoid cell.

Green (1954) pointed out that an internodial cell of *Nitella axillaris* shows a
spiral growth, while the rhizoid cell presents an apical growth. From the present
observation it seems that the rhizoid cell of *Nitella flexilis* scarcely shows a spiral
growth, if at all.

In the previous report the author emphasized the existence of morphogenetic
polarity in the isolated internodial cell or in the cell fragment which was formed
from the internodial cell by strangulation with strips of silk thread. If morpho-
genetic polarity also existed in the rhizoid cell, a new bud would appear at the
apical end of the rhizoid in general. Actually, however, a new bud arose from the
basal end. From this result it seems that the rhizoid has no morphogenetic polarity.
Nevertheless, we are in a position to assume the existence of morphogenetic polarity
in the rhizoid cell by the reasoning that a new bud is formed at the basal end of
the cell in order to grow as a small complete plant consisting of this new bud and
rhizoids, which have already been formed at the basal end of the changed cell, after
the necrosis of the metamorphic cell which is liable to decay soon after shooting
because of full development.

Bonner (1944) mentioned that *Dictyostelium discoideum*, which is a species of
Myxomycetes, is a suitable material for the morphogenetic study because of the
fact that this organism shows only growth at first in its life cycle and its morpho-
genesis occurs after the complete suspension of its growth. The rhizoid cell of
Nitella was metamorphosed into a minor shape of internodial cell after the stoppage
of its growth which had increased in size. The author assumes the rhizoid cell of
Characeae is an excellent material for the observation of morphogenesis in plant cell
having a cell wall and a single nucleus.

From the results of the present work it is likely that morphogenetic ability
remains in an isolated single rhizoid cell just as in the case of the isolated inter-
nodial cell and also that this morphogenetic ability can be activated when the

rhizoid cell is cultured under adequate conditions.

Summary

1. An isolated single rhizoid cell of *Nitella flexilis* was cultured in a medium consisting of 0.8 % agar and a culture solution involving several salts in low concentration both in the dark and in the light. This rhizoid cell continued its growth and showed cell reproduction.

2. When the rhizoid cell was exposed to illumination of fluorescent light of about 30 lux for ten hours a day, the cell which was fully developed was metamorphosed into a small internodial cell. A new bud arose from this metamorphic cell and developed to a small complete form of *Nitella*.

3. An isolated single rhizoid cell of *Nitella* seems to have morphogenetic ability just as in the case of the isolated internodial cell. This morphogenetic ability may be activated under adequate conditions.

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Notes on Some Grasses I

by Tuguo TATEOKA*

館岡亜緒*： イネ科雑記 I

1. Systematic position of the genus Phaenosperma. The monotypic genus *Phaenosperma* including *P. globosum* Munro is distributed in East Asia, and its systematic position was variously discussed. Bentham (1881), Hackel (1887), Bews (1929), a. o. placed this genus in Tristegineae. Pilger (1931) pointed out that this opinion was due to a misinterpretation of the spikelet structure of *P. globosum*. In the same paper, he also maintains the near relationship of this genus to *Sporobolus*.

* National Institute of Genetics, Mishima, Shizuoka Pref.   国立遺伝学研究所

Roschevitz (1937) disposed of *Phaenosperma* as an independent tribe, Phaenosper-meae, in Poatae-Festuciformes. Pilger (1954) assigned Phaenospermeae to Eragrost-oideae. Ohwi (1942) proposed the following classification of Pooideae-Phaenosper-meae: Phaenosperminae-*Phaenosperma* ; Diarrheninae-*Diarrhena* ; Moliniinae-*Molina*, *Moliniopsis, Hakonechloa*. Some information concerning the systematic position of Phaenospermeae, as well as the results of examination of leaf structure in *P. globosum* is presented as follows.

Characteristics of leaf structure in *P. globosum*.——In the epidermis no unicellular or bicellular hair is found. Siliceous cells are round in the lower epidermis, while dumbbell shaped or elliptical in the upper epidermis (Figs. 1 A, B). In the transverse section, mechanical tissues are well developed, and a cell layer which contains a few chloroplasts surrounds the vascular bundles. Assimilative parenchyma is arranged in comb shape, and motor cells are little developed (Figs. 1 C, D).
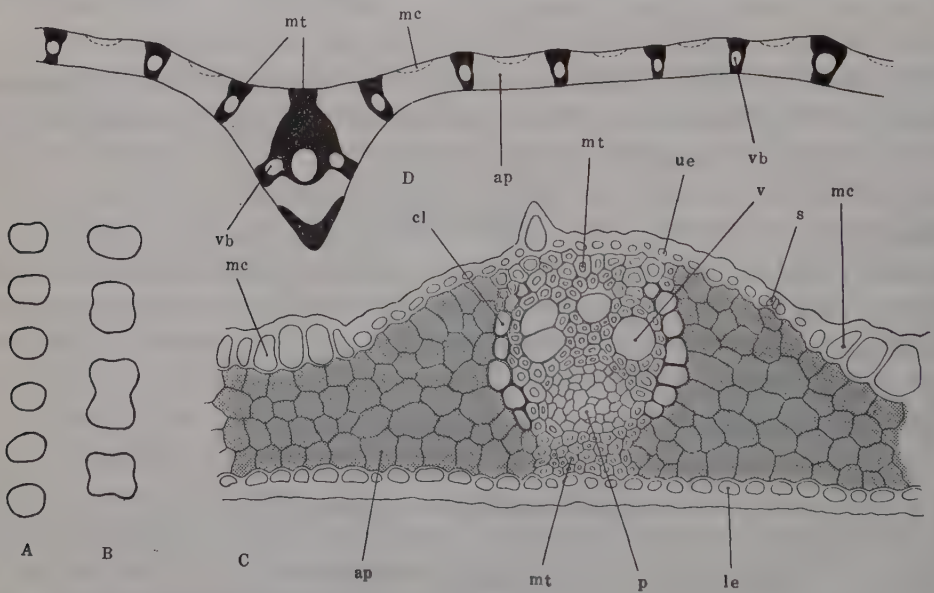

Fig. 1. *Phaenosperma globosum.* A, B. Siliceous cells in leaf epidermis (A—lower epidermis ; B—upper epidermis) ×600. C, D. Transverse leaf section. C ×210, D ×ca.40. ap—assimilative paren-chma. cl—a cell layer which contains a few chloroplasts. le—lower epidermis. mc—motor cell. mt—mechanical tissue. p—phloem. s—stoma. ue—upper epidermis. v—vessel. vb—vascular bundle.

As the description and figures show, the characteristics of leaf structure of *P. globosum* may be regarded on the whole as those of Festucoid type. Lack of bicellular hairs, the round shape of siliceous cells in the lower epidermis and the arrangement of assimilative parenchyma in comb shape do not clearly conform with Panicoid type. Somatic chromosomes of *P. globosum*, according to Avdulov (1931) and Tate-oka (1955), are reported as twenty four, and they are all small. The starch grains of endosperm in *P. globosum* are simple and similar to those of *Uniola* species being large and round (cf. Tateoka 1954, Fig. 14). Lemmas of *P. globosum* have

three thick nerves and one or two other pairs of fine nerves (Fig. 2 c). Large round seeds, 3-lodicules, etc. are specific features of the external morphology of *P. globosum*.
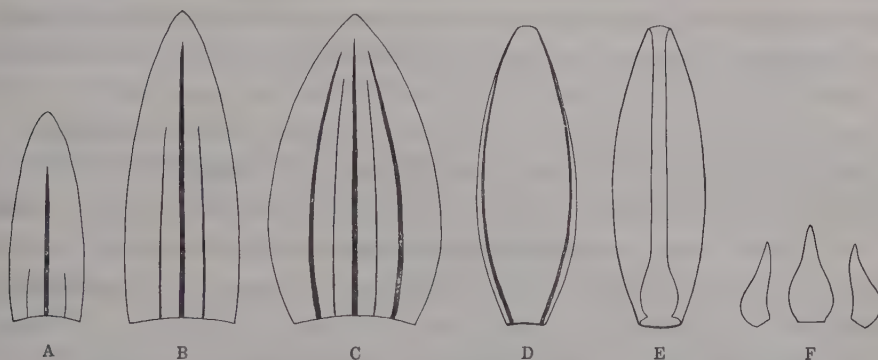

Fig. 2. *Phaenosperma globosum*. A. Lower glume. B. Upper glume. C. Lemma. D,E. Paleae. F. 3 lodicules. × 10.

Characteristics of *P. globosum* described above do not coincide with those of the members included in Eragrostoideae. In leaf anatomical characteristics, the Festucoid type is never found among them. Chromosome characteristics of *P. globosum*, i.e, b=12 (6) and small size, speak aginst referring this genus to Eragrostoideae, and suggests a relationship to some groups of Festucoideae. Pilger (1954) divided the grass family in nine subfamilies. If we follow Pilger's classification, Phaenospermeae must be placed in the subfamily Festucoideae. Considering the characteristics described above, it is the most natural conclusion. Festucoideae may be divided into Eufestuciformes group and the other groups (cf. Tateoka in press (B), then Phaenospermeae clearly belongs to the latter, because the characteristics not only of the external morphology but also of the chromosomes in *P. globosum* do not coincide with those of Eufestuciformes group. Some genera similar to *Phaenosperma* in chromosomal and leaf anatomical characteristics are found in Festucoideae-Non-festucoid genera. Studies of their relationship to *Phaenosperma* will be reported in another paper.

2. **Systematic significance of leaf structure in the genus Garnotia.** The genus *Garnotia* includes 70~80 species which inhabit all over India, South East Asia and New Guinea. The systematic position of this genus has been discussed since a long time. Brongniart (1832) and Steudel (1854–55) placed it in Paniceae, and Bentham (1881) and Hillebrand (1888) assigned its place in Tristegineae. In his excellent paper on *Garnotia*, Santos (1950) notices the fact that such references of *Garnotia* are based on misinterpretation of the spikelet structure of *G. stricta* which represents the type of this genus. The two tribes mentioned above have two florets in each spikelet: a lower reduced floret and an upper fertile one. The same is that of Arundinelleae to which *Garnotia* was referred by Mez (1921). But the spike-

lets of *Garnotia*, which were supposed to consist of one sterile lemma and one fertile floret by Brongniart and others, include only one fertile floret.　Hackel (1887), Bews (1929) a.o. referred this genus to Agrostideae on the basis of having one fertile floret in each spikelet.　Santos (1950), following their opinion, maintains a close relationship between *Garnotia* and *Polypogon*.　Eragrosteae (or Chlorideae) is another group in which the genus *Garnotia* was placed by several authorities, for example by Pilger (1954).

　　Garnotia is a large genus including many species, their chromosomes being never examined and data of leaf structue being scarcely reported.　Avdulov (1931) only indicates that *G. adscencens* belongs to Type II, based on Iakovliev's observation.　The present author examined the characteristics of epidermis and transverse leaf section of *G. stricta* Brongn, and *G. boninensis* Tuyama.　The results obtained are as follows.

　　Epidermis—Both species have threadlike bicellular hairs (Fig. 3, Aa, Ba).　Siliceous cells of *G. stricta* are saddle shaped (Fig. 3, Bb), and those of *G. boninensis* have dumbbell shape (Fig. 3, Ab).　The characteriatics of epidermis of the two species are of Panicoid subtype of Panicoid type.

　　Transverse section—The vascular bundles are surrounded by a cell layer which contains many chloroplasts.　In both species, distinctive cells which contain plenty of chloroplasts and have thick membrane similar to the cells directly surrounding the vascular bundles mentioned above are found scattered throughout the mesophyll.
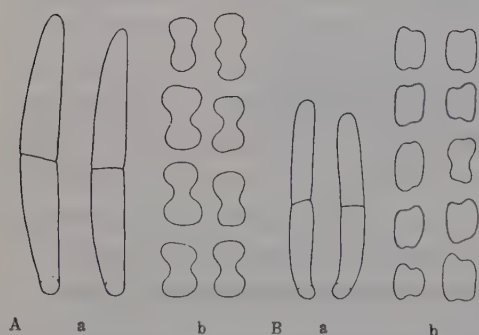

Fig. 3.　Siliceous cells and bicellular hairs.　A. *Garnotia boninensis*.　B. *G. stricta*.　a—bicellular hair, b—siliceous cell. ×375.

Although the species examined are only two, the characteristics of their leaf structure show an interesting feature in respect to the systematic position of *Garnotia*.　Agrostideae and Eragrosteae to which *Garnotia* was assigned by various investigators are clearly different from *Garnotia* regarding the epidermis and the anatomical leaf characteristics, namely Agrostideae shows Festucoid type and Eragrosteae shows Chloridoid subtype of Panicoid type.　The reference of *Garnotia* to such tribes is considered to be erroneous on the basis of the characteristics of epidermis and leaf anatomy.　*Arundinella hirta* Tanaka shares many features with the two examined species of *Garnotia* with respect to leaf structure. Regarding the feature of leaf structure of *Arundinella hirta*, the author prepared another detailed report (in press (A)).　Other members of Arundinelleae have been scarcely examined either histologically or anatomically.　So far observed, the existence of distinctive cells, a characteristic cell layer and Panicoid subtype in epidermal

characteristics are common to *Garnotia* and *Arundinella*, and the existence of distinctive cells indicates their close relationship. While *Garnotia* is clearly different from Arundinelleae in the spikelet structure, much resemblance is found in their habits and in the constitution of spikelets. Considering the findings described above, the genus *Garnotia* may be treated as an independent tribe (Garnotieae) which should be placed near Arundinelleae. But this reqirues further studies of leaf structure in other many species of *Garnotia* and also in other Arundinelleae.

I wish to express my cordial thanks to Dr. J. Ohwi and Dr. Y. Takenaka who gave me various useful advices during the course of the present investigation.

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Miscellaneous Notes on Myriangiales from Japan II***

by Eiichi Kurosawa* & Shigetaka Katsuki**

黒沢英一·*· 香月繁孝 :**　日本産痘瘡病菌 II***

(1)　Elsinoë randii Jenkins & Bitancourt

Phytopath. 28(1): 75–78 (1938); Kurosawa & Katsuki, Plant Prot. 9(9): 367–368 (1955) (in Japanese).

Hab. and distr.: On spp. of *Juglans*. North and South America.

Specimens examined:

On *Juglans regia* var. *orientalis* (Kashikurumi).

Saitama Pref.: Hatogaya, Aug. 21, 1938. E.K. (SK 1062) (IB 5987) (NFC 91092); Aug. 21, 1938. E.K. (KU–K 3); Sept. 15, 1938. E.K. (SK 1063) (KU–K 2);

　*　Nihon Tokushu Nōyaku Co., Ltd. Kurosawa passed away in March 1953. 日本特殊農薬株式会社

　**　Tōa Nōyaku Co., Ltd. 東亜農薬株式会社

***　Kurosawa, E. & S. Katsuki, Miscellaneous notes on Myriangiales from Japan (1) Ann. Phytopath. Soc. Japan 21 (1): 13-16, 1956.

Sept. 9, 1938. E.K. (SK 1064) (KU–K 1).*

On *Juglans mandshurica* var. *cordiformis* (Kurumi).

Tokyo: Hinodai, Sept. 2, 1951. E.K. (SK 1065 & 1066) (KU–K 11 & 12).

Elsinoë randii was described on *Carya illinoensis* from the U.S. and Brazil. This is the first report of the fungus on *Juglans*.

(2) **Sphaceloma bidentis Bitancourt & Jenkins**

Arquivos do Instituto Biologico **10** : 5–6 (1950).

Hab. and distr.: On *Bidens bipinnata* (Sendangusa). South America.

Specimens examined:

Tokyo: Nanao-mura, Oct. 7, 1951. E.K. (SK 1406 & 1407); Bushu, Takiyama, Sept. 25, 1938. E.K. (SK 1408) (KU–K 9).

The present fungus is new to Japan and *Bidens bipinnata* seems to be a new host plant to the fungus. The essential characters of the present fungus coincide with those of *Sphaceloma bidentis*. This was first described on *B. pilosa*.

(3) **Sphaceloma catalpae Kurosawa & Katsuki sp. nov.** (Fig. 1).

Maculis in foliis, rotundis vel leniter irregularibus, numerosis, 0.3–2 mm. diametro, amphigenis plerumque epiphyllis, sparsis vel saepe in prope nervos, sparsis vel

Fig. 1. *Sphaceloma catalpae.* ×1/2
A. Spots on upper leaf surface
B. Conidiophores and conidia (× 750).

coalescentibus, superne pallide sordide brunneis, inferne sordide; acervulis erumpente superficialibus, stromatibus nigro brunneis usque 20–32 μ latis, 16–20 μ crassis; conidiophoris brevis, hyalinis, continuis, 5–15 × 2.5–3.0 μ, ad bases leniter incrassatis, ad apices obtusis vel acutis, ellipticis vel oblongis, hyalinis, 4–8 × 2.0–4 μ.

Hab. and distr.: On *Catalpa speciosa* (Hana-kisasage) (Bignoniaceae).

Specimens examined:

Chiba Pref.: Matsudo, Aug. 9, 1938. E. K. (SK 1060) (KU–K 39); Sept. 12, 1938. E. K. (SK 1061) (KU–K 40) (IB 5986) (NFC 91070).

Tokyo: Hinodai, Sept. 2, 1951. E. K. (SK 1062–Type) (SK 1063 & 1064) (KU–K 41 & 42).

Sphaceloma sp. is reported on *Catalpa bignonioides* in Louisiana, U. S. A. (Arruda, S. C. and C. W. Edgerton, Phytopath, **33**: 1. 1943). (Abs.) and on *C. ovata* in Japan (Jotani, Y., Phytopath. Soc. Jap. **17**(3–4): 168. 1953).

(4) **Sphaceloma picrasmae Kurosawa & Katsuki sp. nov.** (Fig. 2).

Maculis in foliis, petiolis vel caulibus; in foliis amphigenis, paucis vel numerosis, sparsis vel saepe aggregatis, circularibus vel irregularibus, 0.5–1.5 mm. diametro, primum nigris dein pallide brunneis vel griseo-brunneis, centro depressis et saepe perforatis; in nervulis maioribus, petiolis et caulibus, ellipticis vel elongatis, margine leniter elevatis; conidiophoris superne conspicuis, plerumque paucis vel fasciculatis, 3–20, ex stromatis vel mycelio intercellulari, rectis vel plus minusve sinuosis, raro ramosis, continuis vel 1–septatis, atro-brunneis, apice pallescentibus, 9–26 μ longis, 2.6–3.3 μ latis; conidiis fusiformis vel ellipticis, pallide brunneis vel olivaceis, 7.9–15.9 × 2.6–3.3 μ.

Fig. 2. *Sphaceloma picrasmae.* Conidiophores and conidia (×750).

Hab. and distr.: On *Picrasma quassioides* (Nigaki) (Simarubaceae).

Specimens examined:

Ibaragi Pref.: Narado, July 17, 1938. E. K. (SK 1045–Type) (KU–K 43); July 24, 1938. E. K. (SK 1046) (KU–K 44) (IB 5992) (NFC 91084); Sept. 12, 1938. E. K. (SK 1047) (KU–K 45).

(5) **Sphaceloma punicae Bitancourt & Jenkins**

Proc. Sci. Cong. 8 th Washington **3**: 163–164 (1942).

Hab. and distr.: On *Punica granatum* (Zakuro). Italy, South America, and India.

Specimens examined:

Chiba Pref.: Matsudo, Sept. 25, 1939. E. K. (SK 1478) (KU–K 46); Funabashi Aug. 5, 1951. E. K. (SK 1479) (KU–K 47).

Ibaragi Pref.: Itabashi, Aug. 14, 1938. E. K. (SK 1483) (IB 6421).

Tottori Pref.: Tottori city, May 29, 1955. S. K. (SK 1231).

The present fungus is new to Japanese mycoflora.

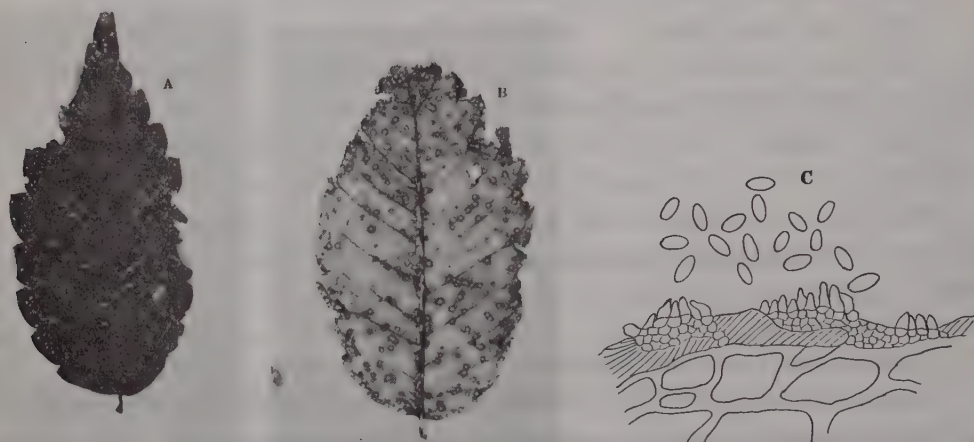(6) **Sphaceloma zelkovae Kurosawa** & **Katsuki sp. nov.** (Fig. 3)

Fig. 3. *Sphaceloma zelkovae.*
A.　Spots on upper leaf surface (×1/2).
B.　Spots on lower leaf surface (×1/2).
C.　Conidial layer (×500).

Maculis in foliis numerosis, sparsis vel aream irregularem coalescentibus, secus nervos locatis, amphigenis, supra conspicuis magis quam infra, circularibus vel irregularibus, minutis, 0.5–1 mm diametro, occasionem usque 2 mm latis, pallde brunneis cum lignum brunneis, dein centro griseis, aliquando perforatis; acervulis erumpente superficialibus, stromatibus usque 25–40 μ crassis; conidiophoris cylindraceis vel conicis, compactis, hyalinis, 2.6–13.2 × 3–4 μ; conidiis oblongo-ellipticis, continuis, hyalinis, 4.5–8.4 × 2.5–5 μ.

Hab. and distr.: On *Zelkova serrata* (Keyaki) (Ulmaceae).

Specimens examined:

Saitama Pref.: Yorii; July 30, 1936. E. K. (SK 1470–Type) (KU–K 48) (IB 5997) (NFC 91090).

Ibaragi Pref.: Itabashi, Tsukuba-gun, May 22, 1937. E. K. (SK 1471) (KU–K 49); June 4, 1938. E. K. (SK 1472) (KU–K 52); June 13, 1938. E. K. (SK 1473) (KU–K 53); June 20, 1938. E. K. (SK 1474); Nogizaki, June 1, 1935. E. K. (SK 1468) (SK 1469) (KU–K 51), Tamura, Aug. 7, 1938. E. K. (SK 1475).

Tokyo: Hinodai, June 25, 1950. E. K. (SK 1476) (KU–K 54); Hachiohji, July 11, 1951. E. K. (SK 1477) (KU–K 55).

Acknowledgement

The Taxonomical Observations on the Natural Variation in *Lilium speciosum* Thunb. II*

By Sadao ABE and Teruo TAMURA**

阿部定夫・田村輝夫：** カノコユリの自然変異と分類学的考察 II*

(Continued)

Classification and Description

The description of the varieties and forms of *L. speciosum* has been made since Siebold and Zuccarini (1838). They were followed by Masters (1872), Baker (1873), Elwes (1880), Tilton (1916), Wilson (1925), Woodcock and Coutts (1935), Woodcock and Stearn (1949) and others. They arranged them for 7 to 10 varieties and forms. The majority of variants were regarded as forms, while Baker or Woodcock and Stearn considered them as ranks of varieties, and Wilson arranged them for both varieties and forms. The descriptions and classifications have been given almost only in Europe, and the bulbs imported from Japan and their clones have been the subjects of them. In Japan no such studies have been made excepting those by Hayata (1911, 1912), in which our Formosa-type was named as *L. Konishii* and *L. Kanahirai*. Wilson (1925) untied Hayata's two species and put them into var. *gloriosoides* Baker of Central Chinese origin, which was regarded as a geographical race of *L. speciosum* by him. A plate of *L. speciosum* var. *gloriosoides* by Watson (1931) can be determined to be the Formosa-type. The Japanese geographical races of this lily found by us differ distinctly from var. *gloriosoides* and can be divided into two main groups, i.e. type II (Koshiki-type) and type III (Nagasaki- and Kôchi-types), which differ each other mainly in the feature of leaves and stigmas, and in growing in isolated regions. They may be distinguished taxonomically and may be placed in varietal ranks respectively, as like as the Formosa-type including the Central Chinese group.

Now, the problem is that to wchich type *L. speciosum* Thunberg belongs. To make clear this point, Thunberg's descriptions (1784, 1811) are very obscure, though "acuta" in the tip of leaves is prevalent in the Koshiki-type. The type specimen is preserved in Thunberg's Herbarium in Uppsala and we could see the photograph of it, through the courtesy of Dr. Maekawa and Dr. Hylander. It is the upper part of a plant with five flowers, in which anthers and stigmata are almost destroyed by insects. Judging from the following points, i.e. the shape of leaves, less impressions of the nerves, and marginal undulation being almost absent, we identify the

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type specimen as the plant of Koshiki-type, and venture to apply the name *speciosum* to the Koshiki-type.

Furthermore, if many plates of *L. speciosum* so far published are examined concerning the shape of stigmata, it is found that the stigmata in the plate in "Kadan Jikinshô" (1694) is truncate, but the figure is too imperfect to be identified, while many plates by Kaempfer in Icones Selectae Plantarum (1791), in "Honzô Zufu" (1828), by Siebold and Zuccarini (1838), as well as in "Yûyô Shokubutsu Zusetsu" (1891) show the Koshiki-type, judging from the shape of stigmata. Accordingly, almost all clones of *L. speciosum* which were grown in Japan at that time, may have been of the Koshiki-type, however, *L. speciosum*, whose flowers bloom early and are paler in shade, is described in "Senka Oden" (1847) and it may probably belong to the Kôchi-type. All the plates of *L. speciosum* under the following names are judged to be the Koshiki-type, from the shape of stigmata, *Kaempferi, tametomo* (Siebold and Zuccarini 1836), *lancifolium roseum* and *album* (Paxton 1838, 1841), *Kaempferi* (Regel 1868), *verum* (Elwes 1880), *Kraetzeri* (Grove 1911) as well as *rubrum, melpomene, album, Kraetzeri* (Yokohama Nursery Co.). Therefore, all the clones which were grown in Europe at that time, may possibly have been of Koshiki-origin, except var. *gloriosoides*. These variants should be regarded as the forms of the Koshiki-type.

In the classification of forms, only rare characteristics should be adopted in order to establish the whole scheme of variation. For this purpose, we will consult Wilson's classification, which has been regarded as the best work. It classifies the forms as follows : f. *rubrum* Hort. (Masters) ; f. *melpomene* Hort. (Elwes) ; f. *roseum* Hort. (Masters) ; f. *punctatum* Courtois ; f. *album* Masters ; f. *Kraetzeri* Duchartre ; var. *tametomeo* Sieb. et Zucc. ; f. *album-novum* Hort. (Mallet) ; f. *magnificum* Hort. ; var. *gloriosoides* Baker. To the white-flowered form, the rank of the form (f. *vestale*) should be rather applied than var. *tametomo*. The f. *Kraetzeri* has been distinguished as a peculiar form having orange pollen from other white-flowered forms, but the pollen of the flowers in Grove's plate (1911) is proved to the orange-brown. The pollen of this shade is rather prevalent, and so f. *Kraetzeri* should not be distinguished from usual white form (f. *vestale*). The f. *rubrum* is not given to especially dark-flowered form and its shade of flowers is usual. The f. *melpomene* is the horticultural variety bred by Hovey through hybridization, and in it no peculiar characteristics can be recognized. Moreover, horticultural varieties should be neglected when we classify botanically, unless they have peculiar characteristics. As f. *magnificum* is of Koshiki-origin, it ought to be included into *speciosum* proper.

Thus, we have classified *L. speciosum* into 3 varieties and 14 forms, including 1 new variety and 9 new forms, as follows :

Lilium speciosum Thunberg in Trans. Linn. Soc., II, 332 (1794), in Mém. Acad. Sci. St. Petersburg, III, 206 (1811). Paxton, Mag. Bot. V. 1, t. (1838). Franchet and Savatier, Enum. Pl. Jap., II, 67 (1879). Elwes, Monog. Lilium, t. 13 (1880). Matsu-

mura, Ind. Pl. Jap., II, pt. 1, 205 (1905). Grove, Lilies, 69 (1911). Tilton in Bailey, Stand. Cycl. Hort., IV. 1870, fig. 2162 (1916). Wilson, Lilies E. Asia, 75 (1925). Woodcock and Stearn, Lilies World, 334 (1949).

I. **Lilium speciosum** var. **speciosum** (ut *L. speciosum speciosum*).

L. speciosum Thunberg in Trans. Linn. Soc., II, 332 (1794), in Mém. Acad. Sci. St. Petersburg, III. 206 (1811). Paxton, Mag. Bot., V, 1, t. (1838). Elwes, Monog. Lilium, t. 13 (1880). Grove, Lilies. 69 (1911).—' *L. superbum* L.' Thunberg, Fl. Jap., 134 (1784).—*Konokko Juri* Banks, Icon. Kaempfer, t. 47 (1791).—*Kanoko Juri* Iwasaki, Hônzo Zufu, XLVIII (1828).—" *L. lancifolium* Hort. Mussche apud Guill Arch. Bot.. 271 (1833)."—*L. speciosum α Kaempferi* Siebold et Zuccarini, Fl. Jap., I, 31, t. 12 (1836)—*L. speciosum lancifolium* Hort. Neumann in Rev. Hort., V, 494 t. (1844).—*L. speciosum* var. *rubrum* Lemair in Fl. des Serr. III, tt. 276-277 (1847).—*L. speciosum speciosum* Masters in Gard. Chron., 1872, 1522 (1872).—*L. speciosum rubrum* Masters l.c.—*L. speciosum* var. *rubrum* Masters ex Baker in Jour. Roy. Hort. Soc. IV, 42 (1873).—*L. speciosum verum* Elwes, Monog. Lilium, t. 13 (1880).—*L. speciosum atro-sanguineum* Hort ex Elwes l.c. pro syn.—*L. speciosum rubrum multiflorum* Hort. Rozen and Son ex Elwes l.c. pro syn.—*Kanoko Yuri* Tanaka & Ono, Yûyô Shoku-butsu t. 946 (1891).—*Aka-kanoko* Yokohama Nursery Co. " Yuri Kasen " (Selected Garden Lilies) (1899).—*L. speciosum Schrymakersi* Hort. Krelage in Jour. Roy. Hort. Soc., XXVI, 363 (1901).—*L. speciosum magnificum* Hort. Wallace in Jour. Roy. Hort. Soc., XXV, lcxxi (1904), Fox in Addisonia VII, 55, t. 252 (1922).—*L. speciosum* var. *purpuratum* Hort. Tilton l.c.—*L. speciosum* var. *macranthum* Hort. Tilton l.c.—*L. speciosum* f. *magnificum* Wilson, Lilies E. Asia, 77 (1925).

Bulb rather flattened globose to globose, brownish yellow (intermediate between 19 and 17′b)* tinged with pink (1″d) on the summit, pink (1″b), dull rose (1′), or reddish purple (1k), some-times brownish yellow, 5-12 cm. in diameter (usually 7-9 cm.); scales thick, rather tightly imbricated, lanceolate-ovate, 5.5-7 cm. long, 2.2-2.7 cm. wide, acute or acuminate, incurved. Stem 0.5-0.9 m. long, erect, terete, rigid, glabrous, green (27″ or 31k), or light brown (9‴i), or purplish brown (71″m or 1‴m). Leaves scattered, phyllotaxis 2/5 or 3/8, 1/2 on the upper part of stem when young, petioled, glabrous, yellowish green (31k), rather leathery, lanceolate to oblong-ovate, 11 to 17 cm. long, 3 to 5 cm. wide, 0.4-0.5 mm. thick, acute, rarely somewhat attenuate, base rounded or broad-cuneate, distinctly 5-7-nerved, slightly impressed along nerves; petiole 0.5-2 cm. long, adpressed to stem. Flowers rose-carmine (somewhat deeper than 71b) to rose-pink (71d), white-margined, or faded gradually towards the margin, sometimes blotched only in the middle, spotted with dark crimson (intermediate between 71k and 71i) to rose (71b), usually sub-pendulous, sometimes sub-erect, lateral or pendulous, fragrant, 1-30 (usually 3-10), racemose; pedicels rigid, spreading and ascending-spreading, 9-11 cm. long, 0.3-0.4 cm. in diameter, upper the shorter, bracts and bracteoles foliaceous; perianth-segments reflexed, slightly waved in the margin, lanceolate to ovate, 6.5-10 cm. long, 2.5-4 cm. (inner segments), 2-2.5 cm. (outer segments) wide, apiculate, lower face with abundant, raised, fleshy colored papillae, without keel, nectariferous furrow light green (31i), yellowish green (27i), greenish yellow (25″b), or light yellow (25f), glabrous; stamens shorter than the perianth, widely divergent, filaments subulate, glabrous, anthers 2-2.5 cm long; pollen chocolate-brown (3k, 5k) or organe-brown (7i); pistil slightly overtopping stamens, ovary 15-25 mm. long, 3.5-4.5 mm. in diameter, style curving slightly thickened upward, stigma capitate. Blossoms in late July to early August. Fruit obovoid-cylindrical, 5-7 cm. long, obtusely

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* Color symbols from " Ridgway's Color Standards and Color Nomenclature ".

angled, summit umbillicate.

Nom. Jap.: Shima-Kanokoyuri (nom. hort., etymologia Shima—insula, Kanokoyuri—*Lilium* cum tepalis punctatis).

Hab.: Kyûshû: Kagoshima Pref.—on the western part of mainland (a part) and on the Islands of Koshiki (abundant). Kumamoto Pref.—on the Islands of Amakusa (rare). Fukuoka Pref.—Munakata and Onga Districts (a part).

1. **Lilium speciosum** var. **speciosum** f. **coccineum** S. Abe et Tamura f. nov.

Flowers crimson (somewhat paler than 71), margined with white, spotted with brodeaux (71k) to dark crimson.—Flores coccinei, argento-marginati, maculis atro-coccineis punctati.

2. **Lilium speciosum** var. **speciosum** f. **roseum** Masters, in Gard. Chron., 1872, 1522 (1872) (ut *L. speciosum roseum*).

L. speciosum var. *roseum* Masters ex Baker in Jour. Roy. Hort. Soc. IV, 42 (1873).—'*L. speciosum punctatum*' Elwes, l.c.

Flowers pink (71f), spotted various shades.

3. **Lilium speciosum** var. **speciosum** f. **album** Masters in Gard. Chron. 1872, 1522 (1872) (ut *L. speciosum album*).

Ryukyu Yuri Iwasaki, Honzô Zufu XLVIII (1828).—*L. speciosum* var. *album* Masters ex Baker in Jour. Roy. Hort. Soc., IV, 42 (1873).

Flowers faint pink. not spotted.

4. **Lilium speciosum** var. **speciosum** f. **vestale** Masters, in Gard. Chron., 1872, 1522 (ut *L. speciosum vestale*). Elws, Monog. Lilium t. 13 (1880).

"*L. Broussartii* Morren apud Mons in Hort. Belg., I, 371, t. 23 (1834)".—*L. speciosum β tametomo* Sieb. et Zucc. in Fl. Jap. I, 31, t. 13 (1836).—*L. speciosum* var. *Album*, Paxton, Mag. Bot VIII, 127 t. (1841).—*L. eximium* Hort. ex Kunth, Enum. Pl. IV, 259 (1843) non Courtois, pro syn.—*L. speciosum* f. *Kraetzeri* Duchartre ex Baker in Jour. Linn. Soc. XIV, 234 (1875).—*Shiro-Kanoko* Yokohama Nursery Co., "Yuri Kasen" (Selected Garden Lilies) (1899).—*L. speciosum Album Kraetzeri* Hort. Krelage in Jour. Roy. Hort. Soc. XXVI, 363 (1901).—*L. speciosum roseum album* P. Barr in Gard. Chron. ser. 3, XXXII, 129 (1902).—*L. speciosum* var. *Kraetzeri* Hort. ex Grove, Lilies, t. 3 (1911).

Flowers pure white, not spotted.

5. **Lilium speciosum** var. **speciosum** f. **album-novum** Mallet in Gard. Chron., ser. 3, XXX, 24 (1901).

L. speciosum punctatum album P. Barr in Gard. Chron., ser. 3, XXXII, 129 (1902). —'*L. speciosum* var. *Kraetzeri*', Yokohama Nursery Co., Illus. Gard. Pl. Jap., II, 5, Fig. 205 (1914).—*L. speciosum* var. *album-novum* Woodcock & Stearn, Lilies World, 339 (1949).

Flowers white, not spotted, pollen yellow (13–15).

6. **Lilium speciosum** var. **speciosum** f. **concolor** S. Abe et Tamura f. nov.

'*L. speciosum* Thunb' Lindley in Bot. Reg. XXIII, t. 2000 (1837).

Flowers crimson, colored entirely, leaving narrow white edge, spotted with dark

crimson.—Flores in toto faciem coccinei, sed angustissime albo-marginati, maculis brunneo-coccineis punctati.

7. **Lilium specioum** var. **speciosum** f. **radiatum** S. Abe et Tamura f. nov.

Flowers white, rayed with rose (intermediate between 71 b and 71 d) to rose pink (71 d) stripes, spotted with various shades.—Flores albi, roseo vel dilute roseo-vittatis, maculis coloribus ornati.

8. "**Lilium speciosum** var. **speciosum** f. **punctatum** Courtois in Cat. Agric. Bot. Gand., 1844, 26" (forsan ut *L. speciosum* f. *punctatum*).

L. lancifolium roseum Paxton, Mag. Bot., V, 267 t. (1838).—*L. lancifolium punctatum* Hort. ex Paxton l.c. pro syn.—"*L. speciosum* var. *punctatum* Marnock in Fl. M., 4, 136, t. 44 (1839)".—*L. speciosum albiflorum* Hooker in Bot. Mag., LXVI, t. 3785 (1840).—*L. punctatum* Hort. ex Lemaire in Fl. des Serr., III, sub tt. 276–277 (1847).

Flowers white, spotted with pink (71 f), pollen yellow.

9. **Lilium speciosum** var. **speciosum** f. **rubro-punctatum** S. Abe et Tamura f. nov.

Flowers white, spotted deep rose (71 i), pollen brown.—Flores candidi, maculis intense-roseis punctati, pollinibus brunneis.

10. **Lilium speciosum** var, **speciosum** f. **compactum** S. Abe et Tamura f. vov.

Stem dwarf, flowers compact.—Planta humilior; flores compacte aggregati.

11. **Lillium speciosum** var. **speciosum** f. **erectum** Walker in Am. Lily Yearbook, 1942, 108.

Flowers suberect to lateral.—Flores suberecti vel lateraliter patet.

The name f. erectum described by Scrase-Dickins (Roy. Hort. Soc. Lily Yearbook, IV, 127, 1935) seems to be derived from the erect character in the stem. Hence, we adopt Walker's name in spite of its later publication.

II. **Lilium speciosum** var. **clivorum** S. Abe et Tamura var. nov.

'*L. speciosum* var. *gloriosoides* Baker' Grove in New Fl. Silva, III, Fig. VII (1930).—'*L. speciosum punctatum*', Wallace in Roy. Hort. Soc. Lily Yearbook, IV, 126 (1935).

Bulb flattened globose, scales rather loosely imbricated, 4–6 cm. long, 1.3–2 cm. wide, lanceolate, acuminate. Stem rather slender, 0.9–1.3 m. long, tending from sub-erect to sub-pendulous, usually nodding at the top. Leaves tending to 1/2–phyllotaxis, subnutant, broad-lanceolate to elliptic-ovate, slightly larger than the type, 12–20 cm. long, 4–6 cm. wide, 0.2–0.3 mm. thick, attenuate, rather dark green (31″m), strongly impressed along nerves, wavy in the margin. Flowers more lightly colored than the type, faded or margined, sometimes blotched, pendulous; blossoms 2–3 weeks earlier than the type; pedicel rather slender, 11–14 cm. long, 0.25–0.3 cm. diameter; perianth–segments much reflexed, much wavy; stigma subcapitately truncate.

Plant growing in Nagasaki is similar to the plant from Shikoku, but differs in the following characteristics.

Bulb globose, but slightly flattened; scales broad-lanceolate; stem 0.6 to 1 m. long, tending to sub-erect; leaves moderate green (31 m). Blossoms 2–3 weeks ear-

lier than the type variety, but later than the plant from Shikoku; pedicels rather rigid, 8–11 cm. long, 0.3–0.4 cm. in diameter.

Folia: phyllotaxis plerumque 1/2, nutantiuscula late lanceolata vel ovato-elliptica, apice gradatim attenuata, nervis valde impressis, margine repanda. Flores; stigmate subcapitao-truncata, patet praecocciore quam typo.

Nom Jap.: Taki-Yuri (nom. vernacul. in Shikoku. Etymologia Taki—cautes, Yuri— *Lilium*).

Specimina typica: Tosayama, Kôchi (S. Abe, Typus in Herb. Univ. Tokyo).

Hab.: Shikoku; Kôchi Pref.—on valleys in the Aki, Kami, Takaoka and Tosa District, and Kôchi City. Tokushima Pref.—on valleys in Naka, Myozai and Mima District (a part), and on the Island of Ôshima. Kyûshû; Nagasaki Pref.—on the western coast of Nishisonogi Peninsula, and on the Islands of Matsushima and Kujûku-shima.

1. **Lilium speciosum** var. **clivorum** f. **metaroseum** S. Abe et Tamura f. nov.

Flowers pink, spotted with various shades.—Flores rosacei, maculis coloribus ornati.

2. **Lilium speciosum** var. **clivorum** f. **vittatum** S. Abe et Tamura f. nov.

Flowers white, rayed with rose to rose pink stripes, spotted with various shades. —Flores albi, tepalis medio roseo-vittatis, maculis coloribus ornati.

III. **Lilium speciosum** var. **gloriosoides** Baker in Gard. Chron., n. s. XIV, 198 (1880). Wright in Jour. Linn. Soc. XXXXI, 134 (1903). J. H. Veitch, Hortus Veitchii, 450 (1906). Watson in New Fl. Silva IV, Fig. LXXVI (1931). Wilson, Lilies E. Asia, 77 (1925). Woodcock & Stearn, Lilies World, 340 (1949).

' *L. speciosum Kaempferi* Sieb. et Zucc.' Hance in Jour. Bot., XII, 262 (1874).— ' *L. speciosum* Thunb.' Franchet in Pl. David., I, 307 (1884).—*L. lancifolium* var. *gloriosoides* Bretschneider, Hist. European Bot. Disc. Chin., 742 (1898).—*L. lancifolium formosanum* J. H. Veitch, l.c. 82.—*L. auratum gloriosoides* J. H. Veitch, l.c. 83.—*L. Konishii* Hayata in Jour. Coll. Sci. Tokyo, XXX, art. 1, 364 (1911).—*L. Kanahirai* Hayata, Icon. Pl. Formosan., II, 146 (1912).

Bulb yellow, flattened globose, scale lanceolate-ovate, 2.2–2.5 cm wide. Leaves lanceolate to oblong-ovate, 12–20 cm. long, 0.45–0.5 mm. thick, scarcely impressed along veins. Flowers white, largely blotched with crimson to the center or spotted crimson to dark crimson; perianth segments much reflexed, wavy, raised fleshy papillae numerous, nectariferous furrow relatively long, green (29 k); stigma sub-capitately truncate; sub-constrict; blossoms later than the type.

Nom. Jap.: Taiwan-Kanokoyuri.

Hab.: East central China; Lushan mountains, Kiangsi Province. Formosa; Sekitei, Heirimbi and Dandaigai, Taihoku Province.

1. **Lilium speciosum** var. **gloriosoides** f. **sanguineo-punctatum** S. Abe et Tamura f. nov.

Flowers white, spotted with crimson, pollen brown.—Flores albi, maculis sanguineis punctati, pollinibus brunneis.　　　　　　　　　　　　　　　*(To be continued)*

イソニガナの染色体について

竹 本 貞 一 郎*

Teiichirô TAKEMOTO*: On the chromosomes of *Ixeris nipponica* Nakai

ニガナの倍数性には本邦山野に広く分布するニ
ガナの 3 倍性，高山産クモマニガナの 4 倍性，高
山産タカネニガナの 2 倍性があり，それらの染色
体数並びに生殖方法の研究結果は岡部博士により
すでに報ぜられている(1932)。これら植物の核型
研究の結果は筆者により報ぜられた（竹本 1952,
1953, 1954)。今回さらに海岸産イソニガナの生殖
法並に核型の研究を行つたのでその結果を報告す
る。

材 料 と 方 法

Ixeris nipponica Nakai (*Lactuca nipponica*
Nakai)　イソニガナは中井猛之進 (1920) によつ
て記載された越後国柏崎地方海岸の
特産種**で日本海に面する水面に近
く潮吹のかゝるあたり洪積層の赤褐
色粘土層にダイモンジソウなどと共
に群生しまた波打際から少しはなれ
た海岸の雑木林の小路のほとりなど
にも点々と生えているのが認められ
る (Fig. 2)。

1953 年秋, 柏崎海岸に自生するイ
ソニガナの幼苗を本教室の圃場に移
植したものを研究材料として用い,
生殖法の実験及び染色体の観察は素
焼鉢に栽培したものについて 1954
年 5-6 月に行つた。鉢植にした植物
の根端細胞が染色体の観察に供せら
れ, 処理及び固定染色は TJIO &
LEVAN (1950) の方法に幾分変更を
加えた方法を用いた。やゝ長めに切

りとつた根端を液温 18–20 °C において 0.002
mol 8-オキシキノリン水溶液に 30–50 分間浸し
50–60 分流水洗ののち 45% 醋酸液で固定し 1N-
HCl で加水分解処理を 行つて スライドグラスに
のせ醋酸オルセイン液を注ぎ軽く押しつぶしてプ
レパラートをつくる。

核型分析結果の現わし方として次の方法を用い
た。まず個々の染色体についてその長さを測定し
その全染色体の長さの総計に対するパーセントを
以て各染色体の長さ (relative length) を示し,
各染色体については短腕の長腕に対する比を指標
(index) として核型を示した。かくして 5 個体に
ついて各個体の根端細胞で見られた全染色体の測

Fig. 1.　*Ixeris nipponica* Nakai イソニガナ
(Photo. May 23, 1954).

* 岡山大学教育学部生物学教室
** 或は他の地方にも分布するかも知れない。

定値の平均値を示したのが第1表であり，この表にもとづいて第4図をつくつた。第4図の図表のＸ軸は染色体の相体的な長さを，Ｙ軸は指標をあらわす。

この表わし方は Tjio & Hagberg (1951) の試みているものであるが従来のものに較べて優れていると思われるのでこれを採用した。

<h3>実 験 と 観 察</h3>

イソニガナの生殖方法を明らかにするために交配実験を行つた。まず本種が単為生殖を行うか否かを確かめるために総苞で閉ざされた若い花蕾をカミソリで横断して未熟の葯と柱頭の部分を除去し，なお完全を期して硫酸紙袋でおゝつた。この除葯及び除柱頭実験に用いられた頭状花数 26，その結果は1粒の結実も見られなかつた。次に同株の頭状花間の人工自家受粉を 45 箇の頭花に施したものは全く結実がなく，これに反して異株間の受粉を行つた 50 箇の頭花はいずれも よく結実し種子稔性並に種子発芽率が高いという実験結果が得られた。

根端細胞において 14 箇の染色体がみられる。この 14 箇の形態を精細に観察すれば長さ及び狭さくの位置において2箇ずつが相似ており全染色体は 7対にまとめられる （Fig. 3, I-VII）。

狭さくの位置によつて定められる長短腕の比を精密に測定した結果は第1表及び第4図にあらわされている。最大の長さをもつ第1対染色体においては2狭さくがあり，その1つは次中部に他は次端部の位置にある，後者によつて最短腕が形成されている。第 II-VI 対の染色体ではそれぞれ概して次中部に狭さくがあり，最小の第 VII 対では次端部にある。

各染色体の実長，相対的長さ並びに長短腕の比は第1表に示される。第 3, 4 図及び第1表によつてみれば総数 14 箇の染色体のうち相対応する2箇は形態的に殆んど差異が認められない。

<h3>考 察</h3>

今日までのニガナに関する細胞学的研究には次のものがある。*Ixeris dentata* Nakai （ニガナ）及び *Ixeris dentata* var. *octoradiata* Nakai （ハナニガナ）はいずれも本邦各地の原野山麓に広く分布し単為生殖の方法によつて種子をつくり繁殖しており染色体数 $2n=21$（岡部 1932, 竹本 1952）。これらはいずれも同質3倍性に近いものであるが純然たる同質ではなくやゝ変化した染色体構成をもつものであることは さきに 報ぜられた （竹本 1952, 1954）。また *Ixeris dentata* subsp. *alpicola* Kitamura （*I. alpicola* Nakai, タカネニガナ）は八ヶ岳，白馬岳，日光その他の高山産，染色体

Fig. 2. Distribution of *Ixeris nipponica* in the Kashiwazaki littoral, Echigo, Japan.

数 $2n=14$ で有性生殖を行う2倍種であり，また *I. dentata* subsp. *Kimurana* Kitamura （クモマニガナ）は $2n=28$ で単為生殖を行う4倍種である（岡部）。これらニガナ各種の染色体各組は互にほゞ相似た形態を具えている （竹本 1952, 1953, 1954）。

イソニガナの染色体数及び核型については，これまでに報告がなく筆者の研究によつて $2n=14$ を有する2倍体であることがわかつた（竹本 1953, 予報）。 中井博士の分類によれば，イソニガナは *Ixeris nipponica* なる種名を設けて *Ixeris dentata* とは別な種として取扱つてあるが，核学的見地よりすれば *I. dentata* なる種に包含されている各種とこの *I. nipponica* とは染色体の形状，大きさにおいて互に類似した構成をもつている。

イソニガナの生殖方法を確かめる実験結果から本種は明らかに有性生殖を行つて種子をつくること，また異株間の交配ではよく結実し，同株間の受粉では結実しないから自家不和合性であり，除

Fig. 3. Serial alignment of somatic chromosomes in a root tip cell
of *Ixeris nipponica*, treated with hydroxyquinoline. ×2400.

Table 1. Measurements of the chromosomes of *Ixeris nipponica* Nakai
(average values, in μ).

Pair No.	Member 1						Member 2					
	Long arm	Short arm	Short-est arm	Total	Rel. length	Index	Long arm	Short arm	Short-est arm	Total	Rel. length	Index
I	3.5	2.9	1.2	7.6	9.4%	0.83	3.5	2.9	1.2	7.6	9.4%	0.83
II	3.7	2.6		6.3	7.9	0.70	3.7	2.6		6.3	7.9	0.70
III	3.8	2.2		6.0	7.5	0.58	3.8	2.1		5.9	7.4	0.55
IV	3.1	2.8		5.9	7.4	0.90	3.1	2.7		5.8	7.2	0.87
V	3.0	2.6		5.6	7.0	0.87	3.0	2.5		5.5	6.9	0.83
VI	2.8	2.2		5.0	6.2	0.79	2.8	2.2		5.0	6.2	0.79
VII	2.8	1.0		3.8	4.7	0.36	2.8	1.0		3.8	4.7	0.36

Lengths of the chromosomes being somewhat contracted through hydroxyquinoline.
The shortest arms are not included into the values of index.

葯及び除柱頭実験結果によつて単為生殖を行わないことが確認された。

3倍体である普通種ニガナ及び4倍体であるクモマニガナが2倍体のタカネニガナ或はイソニガナから派生したものであるかどうかについては, 外部形態と核型にのみ基いて即座に結論することはできないことであるが, これら2倍, 3倍及び4倍種の染色体各組の間には互に相似た形態が認められるから, 核型の見地よりして或はいずれも同一の原始型から由来したものではあるまいかと推察される。従来知られた2倍種のニガナとしては高山産のタカネニガナに限られていたが, 平地に産するイソニガナの存在が判明したことは, この考察の可能性を一層強めるものであると思われる。

またニガナ類数種の植物中, 本邦に分布の広い3倍種及び高山性の4倍種が単為生殖を行いこれに対して2倍性のタカネニガナ及びイソニガナ両種のみが有性生殖を行つていること, 2倍性ニガナが高山という特殊な地域でなくて海岸に自生することから考察して, イソニガナがニガナの原始型に近い植物ではあるまいかという暗示が与えられる。

終りに御懇篤な指導を賜わつた下斗米教授に深謝し, 材料の蒐集に御厚意を寄せられた矢野孝二, 吉川純幹の両氏並びに研究の援助を得た山田義隆, 佐藤博文の諸君に謝意を表す。

Résumé

1)　In this study the examinations of the mode of reproduction and the observations of the morphology of somatic chromosomes of *Ixeris nipponica* Nakai (Nom. Jap. *Isonigana*) growing in the Kashiwazaki littoral, Echigo, Japan were made.

2)　It was confirmed that the mode of reproduction of the species is sexual, and not apomictic.

3)　Zygotic chromosome number is $2n=14$.

4)　The fourteen chromosomes consist of two sets which are morphologically similar to each other. The pair of the largest chromosomes have two constrictions; one is submedian and the other subterminal, while the remaining six pairs of chromosomes have only submedian or subterminal constrictions respectively. Concerning shapes and sizes, the set of chromosemes of this species resembles the set of triploid and tetraploid species of *Ixeris dentata* Nakai.
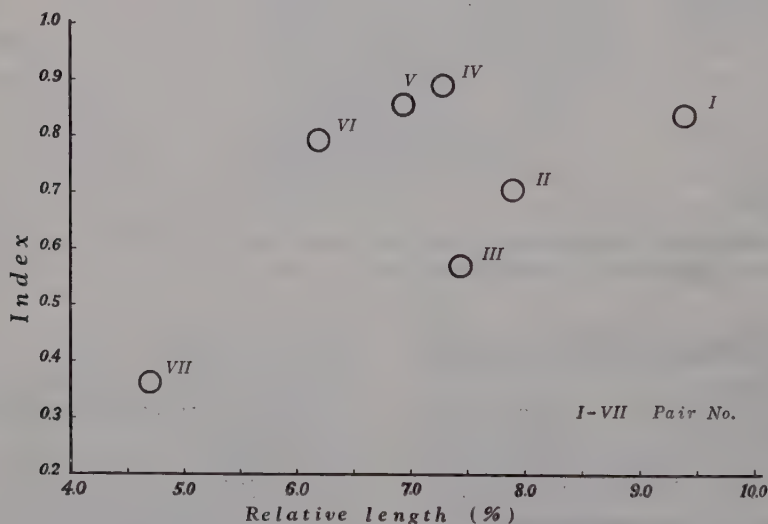

Fig. 4.　Diagram showing the average values for the relative chromosome length (X-axis) and index of short arm on long arm (Y-axis) of the karyotype of *Ixeris nipponica*.

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本 会 記 事

支 部 通 信

関東支部

6 月例会（6 月 16 日，於東大理，植物講義室）幾瀬マサ：日本植物の花粉について．小倉安之：カタラーゼと余談．

九州支部

支部大会（5 月 26，27 日，於九大農，理，日本動物学会，日本生態学会と合同）植物学関係．特別講演．野口彰：蘚類の分布と地質との関係．一般講演．楠元司：冬期における常緑広葉樹の光合成生産．藤野正義：ツユクサの気孔開閉運動に及ぼすエネルギー代謝阻害剤の影響．小野孝：苔類の糖について．石川重夫：種子の発芽に及ぼすクマリンの作用（続報）．松下亀久：タマネギの根の呼吸（第 3 報）．稲田朝次：ツツジの花のモザイクに就いて．小野林，小長光与壮*：葉におけるacid phosphatase 作用について．山下知治：二・三の植物体に於ける同化生成物質の消長に及ぼすカリウム（供給量）の影響．茅野博：ホウチャクソウにおける染色体相互転座．渡辺泰州：腕相同染色体の螺旋の方向．三宮正信：二・三蘚類の染色体数について．井上覚：ノコンギク（*Aster ageratoides* subsp. *ovatus* Kitamura）の 6 倍性天然雑種に関する細胞学的研究．服部新佐，桑原幸信*：*Calypogeia sendaica* Steph.（苔類）について．岩政定治：トロイブゴケ属（*Treubia*）の種の検討．宇佐美和夫：切枝の水没部の剥皮による水分関係の変化．長田武正，尼川大録*：対馬島蘚苔フロラの植物地理学的・生態学的考察（予報）．須股博信*，佐藤仁蔵，二村昭八，鈴木時夫：スダシイ群団の山地性の一群集について．清水正元：肥料の種類及び肥沃度とメヒシバの生育並びに体成分の変化．鈴木時夫：巨視的微視的にみた植物社会の環境．

第 40 回例会（6 月 23 日，於九大教養部）小谷信矢：着生植物の樹上分布と日補償点．中村和郎，渡辺皓：遺伝学新文献抄読 I, II.

熊本例会（第 1 回）（6 月 23 日，熊大教養部）八戸正夫：ユキノシタの葉の細胞の原形質分離及び修酸石灰の結晶について．木通邦武：スイゼンジノリについて．展示実験．石川重夫：簡単な植物実験．井上覚：体細胞染色体の見方．

本会会員　堀川富弥氏は昭和 31 年 3 月 25 日死去されました。

本会会員　細井暁光氏は昭和 31 年 6 月 21 日死去されました。

ここに報告し謹んで哀悼の意を表します。

日 本 植 物 学 会

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1)　投稿は会員に限ります。

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（例）　Bot. Mag. Tokyo **69**: 192 (1956)

Journ. Fac. Sci. Univ. Tokyo III. **6** (1): 1 (1954)

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　　　　東京都本郷局区内

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　　　　　　日本植物学会編集幹事

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特 に 欧 文 原 稿 に つ い て

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The Taxonomical Observations on the Natural Variation in *Lilium speciosum* Thunb. III[*]

by Sadao ABE[**] and Teruo TAMURA[**]

阿部定夫[**] ・ 田村輝夫[**]： カノコユリの自然変異と分類学的考察 III[*]

(*Continued*)

Discussion

Each of the three Japanese geographical races of *L. speciosum* grows wild in the different regions and seems to have varied separately. Other examples of geographical variants of the genus *Lilium* in Japan are as follows; *L. auratum* Lindl. var. *platyphyllum* Baker against *L. auratum, L. japonicum* Thunb. var. *albomarginatum* Koidz., and *L. japonicum* var. *abeanum* Kitam. against *L. japonicum*. Concerning the relation between the species given different names, the following examples seem to be geographical races, i. e. *L. bukosanense* Honda against *L. maculatum* Thunb., as well as *L. talanense* Hayata against *L. callosum* Sieb. et Zucc. It comes into question, whether each geographical race or *L. speciosum* came into existence as a result of the development of original characteristics by chance or owing to the effect of ecological conditions. To make clear this point, first we will investigate the characteristics of stem and leaves of three Japanese races. The form of the plants in the wild is generally erect in Koshiki, sub-erect in Nagasaki, and sub-erect or sub-pendulous in Kôchi. As mentioned above, the form of the plants seem to be affected by environmental factors, and the sub-erect or sub-pendulous plants are apt to be found on the cliff. As the wild habitats in Nagasaki or Kôchi are mainly the cliff, so that the phyllotaxis of the plants of both origins are apt to become 1/2 in order to receive sunlight, and the plants to be sub-erect or sub-pendulous; and these may be regarded as the cases of adaptive variation.

The stem is short and thick in the Koshiki-type, long and thin in the Kôchi-type, and intermediate in the Nagasaki-type. The leaves are large and thin in the Kôchi-type, rather small and thicker in the Koshiki-type, and intermediate in the Nagasaki-type. These characteristics seem to have been developed under the environmental conditions of the wild habitats. In Kôchi, there is more rain-fall and the habitats are in valleys and surrounded by forests in less sunlight and in much

humidity ; while in the Islands of Koshiki there is less rain-fall, and the habitats are on grass land in much sunlight and in rather dry.　On the other hand, in Nagasaki, the habitats are mainly on islands, along the edge of the forest, and are less sunny than those in Koshiki.　Such direction of the above mentioned variations in the Koshiki-type suggests the differences between the inland form and the coastal form, and this variation may be regarded as the case of the adaptive one.　Some examples of this sort of variation within species are as follows : *Setaria viridis* Beauv. to *S. viridis* f. *pachystachys* Makino (in shortening of stems); and *Viola mandshurica* W. Becker to *V. mandshurica* var. *boninensis* (Nakai) Mizushima, and *Paederia chinensis* Hance to *P. chinensis* var. *maritima* Koidz. (in thickening of leaves).　The pendulous tendency of the leaves in the Kôchi-type seems to be due to the thin texture of larger leaves.

Among the characteristics ot flowers, the time of flowering seems to have been influenced by the enviromental factor of the wild growing region.　The ripening of seeds of *L. speciosum* requires usually 85 to 90 days after flowering.　The earlier the winter visits, the more difficult the ripening of seeds becomes.　Therefore, in the less warm region, only those plants which bloom early may have successors. So the three types bloom in the following sequence, the Kôchi-type, the Nagasaki-type and the Koshiki-type : the temperature of each of the three regions is higher in the same sequence mentioned above.

Various characteristics mentioned above are important to distinguish the three types of Japanese geographical races, and these geographical races may be regarded as the ecotypes.　Examples of the ecotype in the Japanese *Lilium* may be *L. bukosanense* against *L. maculatum* and *L. auratum* var. *platyphllum* against *L. auratum*. *L. bukosanense*, which grows on the cliffs of Mt. Bukô has sub-erect stems, seems to be a mountain form of *L. maculatum*.　The variation of *L. auratum* var. *platyphyllum* growing in the islands of Izu from *L. auratum* growing in the mainland of Honshû seems to be due to the isolation caused by the fossa-magna and also to the maritime climate of the islands.

The variation of characteristics, seemingly not influenced by ecological factors, are as follows : the leaves in the tip-form and impression in veins, the stigmata in their shape and the flower-segments in the deepness of shades and coloring types. However it is possible that the deep shade of flowers is apt to appear in a warmer climate.　The characteristics of the Nagasaki-type are rather similar to those of the Kôchi-type, especially in leaves and stigmata, therefore, both the types seem to be closely related each other.　Accordingly, from the evolutional point of view *L. speciosum* can be divided into the three main groups, the Formosa-type, the Koshiki-type, and the Kôchi-type, with Nagasaki-type, and the differentiation of these three groups may probably be older than that of the Kôchi-type and the Nagaski-type.　Now, comes into question how these ecotypes have differentiated across the sea.

The way of the present distribution of *L. speciosum* has been obscure. One way of the distribution across the sea may possibly be that this lily was carried by the Black Current from the Continent of China to our country. This seems to be proved by the fact that the regions of the wild growth in Japan are chiefly on the coasts facing to the southwest; and the possibility of the distribution of the lily by a current was asserted by Stoker (1933). But the reason is not made clear why there is no wild growth of *L. speciosum* in the Loochoo Islands lying between Japan and the Continent of China and it cannot be also overlooked that these ecotypes established across the sea differ distinctly each other. The Japanese Archipelago was a part of the Chinese Continent, in the Palaeogene, in which the present growing regions of Kôchi and Tokushima were probably on the coast (Watanabe 1933), and it is possible that *L. speciosum* was spread through land. Considering the remarkable differences between the Formosa-type and the Japanese types and the differentiations of the ecotypes in Japan, the present types seem to have been established in very old days. From the geohistorical point of view, Maekawa (1949) called the imagined old peninsula from the Chinese Continent Makinoesia-Region, which contained all the Japanese Archipelago and the present Japan Sea and existed through the Eocene and the Oligocene. The Makinoesia-Region is referred to as the base of Japanese floristic zone, and he suggested that the region might be divided into the Western Makinoesia and the Northern Makinoesia, and that the former floristic zone should be related to that of the Old Yangtseking Region and the latter to that of the northern part of the continent. The distribution of lilies in Japan is connected directly to that in the Continent. Namely, *L. dauricum* Ker-Gawl., *L. medeoloides* A. Gray growing in Hokkaido and the alpine regions of the middle part of Honshû, are also found in Saghalien, Kamchatka, the Maritime Province and Manchuria. *L. Leichtlinii* Hooker var. *Maximowiczii* Baker and *L. concolor* Salisb., which are found in Honshû and Kyûshû, are distributed in Korea and Manchuria, the latter extending to Central China. *L. callosum* Sieb. et Zucc. is distributed in Kyushu, Loochoo, Formosa, the Central China, and also in Manchuria and Korea. As to the connection of the distribution of Japanese lilies to that of the continental ones, the northern species extend to the northern part of the Continent and the southern species to the southern part of the Continent. *L. speciosum* has the similar distribution to *L. callosum*. It is supposed from the pattern of the distributions of the lilies mentioned above, that they had moved before the Japanese Archipelago was isolated. As the distributional areas of the Japanese ecotypes in *L. speciosum* are separated by the seas, their differentiation will go back to the Late Pliocene, in which the wild growing region of each ecotype was separated (Watanabe 1933), if the isolation of the areas was induced by the division of land. Considering the close resemblance of the Nagasaki-type to the Kôchi-type, the differentiation into these types and the Koshiki-type may be older than that into the Nagasaki-type and the Kôchi-type. The courses of this evolution is obscure. How-

ever, it may be supposed that *Lilium* of Japan originated in the Continent, where many species are found, and that the Chinese form of *L. speciosum* is its precursor as asserted by Grove (1930). Investigating the affinity of *L. speciosum* with the other lilies in the Chinese Continent, we find several species which seem to be related to *L. speciosum* in respect of the feature of leaves and the shape and color of flowers: i. e. *L. Wardii* Stapf. in Tibet, *L. taliense* Franchet, *L. Duchartrei* Franchet, *L. lankongense* Franchet in the West China (Fig. 9). It is highly probable that the place of the origination of the prototypes or a series of prototypes of these lilies including *L. speciosum* was in the mountain region of the West China. As to *L. speciosum*, its wild growing regions are much isolated in the Central China and Formosa. This fact shows that the area of the distribution of *L. speciosum* once expanding far and wide was divided in the course of time. Accordingly, *L. speciosum* may be one of the old races, and it is highly probable that its originating place was in the Chinese Continent. It is believed that the north-eastern part in the old area of the distribution of *L. speciosum* has remained in Japan with the gradual changing of the plant and became the center of the distribution. Among these ecotypes, the Koshiki-type is the strongest and has the highest variation, which promises future luxuriance. The prototype of *L. speciosum* which originated
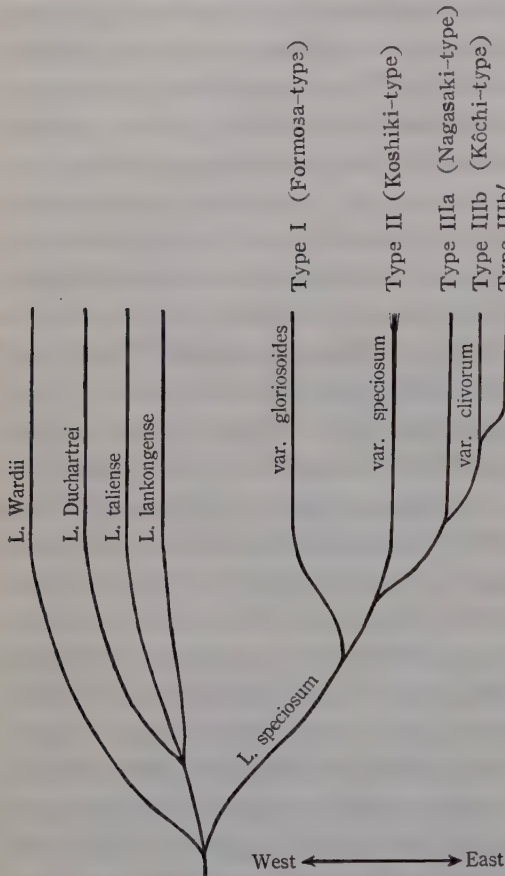

Fig. 9. Imagined courses of speciation in *Lilium speciosum* group.

in the Chinese Continent and moved eastward, may probably have been turned into the Nagasaki- and Kôchi-types, through the Koshiki-type or its prototype, at the border of the distribution area. The former types may have been weakened and isolated on the cliffs, partly owing to the development of forests. On the other hand it is supposed that the Koshiki-type, which has remained in the south-western part of Kyûshû, has been most adapted to the climate and has thrived and produced many variations. Consequently these variations may have still more strengthened the race itself. Thus, it may be concluded that the Koshiki-type plays the leading part in the evolution of *L. speciosum* and the Nagasaki- and Kôchi-types may be

side branches (Fig. 9).

As the reasons for which *L. speciosum* is apt to grow in the isolated regions, the following points may be pointed out. First, *L. speciosum* requires sunshine for its growth. It grows and propagates well in a sunny place, but does not grow in a forest. This is affirmed by the facts that this lily grows vigorously after burning the surrounding grass and was eradicated owing to the development of forests. But, growing in a less thick forest, *L. auratum* distribute rather continuously. *L. speciosum* is regarded to be a precursor in vegetation from the respects mentioned above, and to be unable to thrive in the Eastern Asia in which forests develop easily.

Second, *L. speciosum* is adapted to the warmer climate.

Third, the adaptability of this lily to soil is narrow. *L. speciosum* grows generally in the clayey soil, into which rocks have decayed, and does not grow in the volcanic soil. On the contrary, *L. Leichtlinii* var. *Maximowiczii*, which is adapted to various kinds of soil and even to the volcanic soil, spread widely. Thus, *L. speciosum* is climatically adapted to the coastal region of Kyûshû and Shikoku, and remains on the cliffs or islands, where sunshine is got easily. There are some regions where *L. speciosum* was found in abundance, in its habitat a short time ago but is not seen now. This may be due to the excessive collection of the beautiful flowers.

Although the history of cultivation of *L. speciosum* is obscure, the use of it as the ornamental plant may have probably begun in considerably old times in Japan, refering to the descriptions in old literatures. In Japan, the wild plants of *L. speciosum* were probably transplanted in ancient times in many wild growing regions. The Koshiki-type, which has been the leading cultivated form, may have been taken from the Islands of Koshiki or from the past wild growing regions in the southern part of Kyûshû. The reason why the Koshiki-type has been particularly developed into the horticultural plant may be that it was selected for its many good characters because all the ecotypes were once grown with the probable exception of the Nagasaki-type. Both the Kôchi- and Formosa-types are relatively weak and difficult to cultivate, but the Koshiki-type is strong, polymorphic and is adapted to horticulutural purposes, and has also the most beautiful flowers of all the types of *L. speciosum*.

The wild plants of *L. speciosum* have differenetiated into some ecotypes, one of which we have cultivated for ornamental purposes, using wild bulbs even nowadays. Older horticultural varieties of the ecotype were derived from the natural variation and can be found in the wild state now, but on the other hand, newer horticultural varieties have been successively improved nowadays mainly through crossbreeding in Europe and America, and it is very interesting to be able to trace the derivation of a modern crop from the wild plant.

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* Those mentioned in the text were excluded.

Notes on Some Grasses II

by Tuguo TATEOKA*

館岡亜緒*:　イネ科雑記　II

3. **Distinct characteristics of Arundineae from Eragrosteae**.　Although a large majority of Eragrosteae are clearly distinct from Arundineae in external morphology, it is difficult to determine whether several genera should be treated as members of Eragrosteae or placed under Arundineae.　C. E. Hubbard (1936) assigned the genera *Neyraudia, Cleistogenes, Crinipes* and *Gouinia* to Eragrosteae, while Pilger (1954) referred them to Arundineae.

Tateoka (in press) examined the leaf anatomy in six genera of Arundineae (*Arundo, Phragmites, Molinia, Moliniopsis, Hakonechloa* and *Cortaderia*) and found the following features: vascular bundles are surrounded by a cell layer which does not contain chloroplasts.　Parenchymatous cells often bear cell wall processes and chloroplasts are uniformly distributed throughout the mesophyll.　Though Prat (1936) referred the anatomical characteristics of *Arundo* and *Phragmites* to Panicoid type, they are somewhat different, because the typical Panicoid type, shared by a large majority of Panicoideae and Eragrostoideae, has a cell layer directly surrounding the vascular bundles which contains many chloroplasts.　When we follow Avdulov's classification, the characteristics of Arundineae mentioned above belong to Type II, while those of Eragrostoideae and Panicoideae belong to Type I.　Also, Arundineae and Eragrosteae are different in the epidermal characteristics; Arundineae shows Panicoid subtype, while Eragrosteae Chloridoid subtype.

With respect to leaf structure *Neyraudia* is intermediate between Eragrosteae and the members of Arundineae indicated above.　The results of the author's observation of leaf structure in *Neyraudia reynaudiana* (Kunth) Keng are as follows: vascular bundles are surrounded by a cell layer which contains large amounts of

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* National Institute of Genetics, Mishima, Shizuoka Pref. 国立遺伝学研究所

chloroplasts (Fig. 1, C), threadlike bicellular hairs are found on the epidermis (Fig. 1, A), siliceous cells are rectangular (Fig. 1, B) and no cell wall processes could be found. As described above, the anatomical characteristics of Neyraudia are the same as those of Eragrosteae, and in the epidermal characteristics Neyraudia resembles Arundineae. Chromosomal characteristics of this genus have not

Fig. 1. *Neyraudia reynaudiana* (Kunth) Keng. A, Bicellular hairs found on the epidermis. ×500. B, Siliceous cells of epidermis. ×500. C, Transverse leaf section. ×500. chl–characteristic cells which contain many chloroplasts. le–lower epidermis. mc–mechanical cells. mtc–motor cells. ue–upper epidermis. vb–vascular bundle.

been clarified. The systematic position of *Neyraudia* is still uncertain, but considering that the characteristics of transverse leaf section are more variable than the shape of bicellular hairs and that threadlike bicellular hairs have never been observed in the members of Eragrosteae, the genus *Neyraudia* may be included in Arundineae and should be probably treated as an independent subtribe separated from typical Arundineae.

4. Peculiar features of leaf structure in the genus _Thysanolaena_. The genus *Thysanolaena* including only one species, *T. maxima* O. Kuntze, occurs in tropical Asia and South America. Bentham (1881) and Hackel (1887) placed this genus under Tristegineae, and Camus and Camus (1922) referred it to Paniceae. On the other hand, Stapf [after C. E. Hubbard (1934)] is of the opinion that it may fall within Arundineae. C. E. Hubbard (1934) proposed a separate tribe,
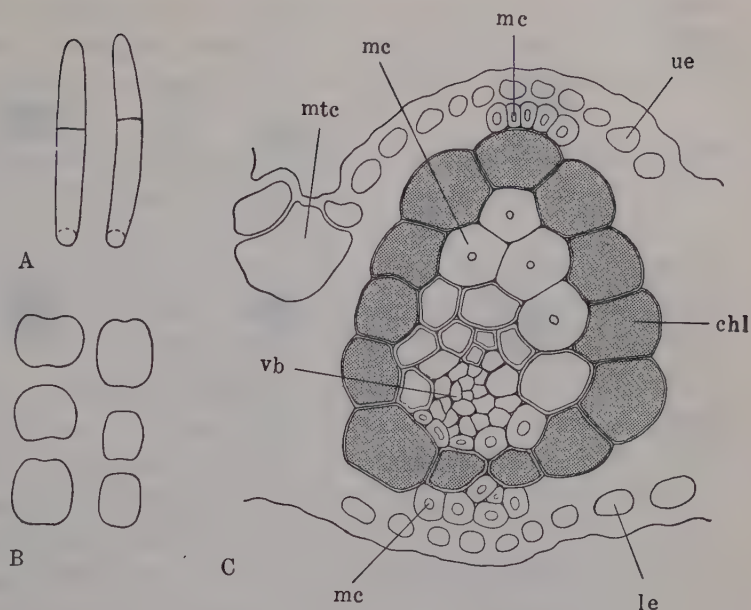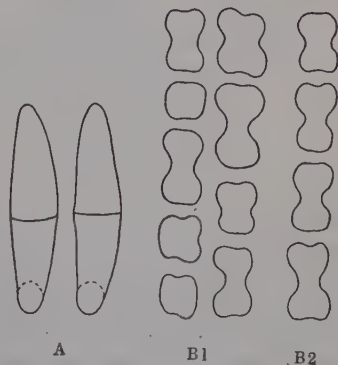

Fig. 2. *Thysanolaena maxima* O. Kuntze. A, Bicellular hairs found in the lower epidermis. ×600. B, Siliceous cells of epidermis, 1–upper epidermis, 2–lower epidermis. ×ca. 400.
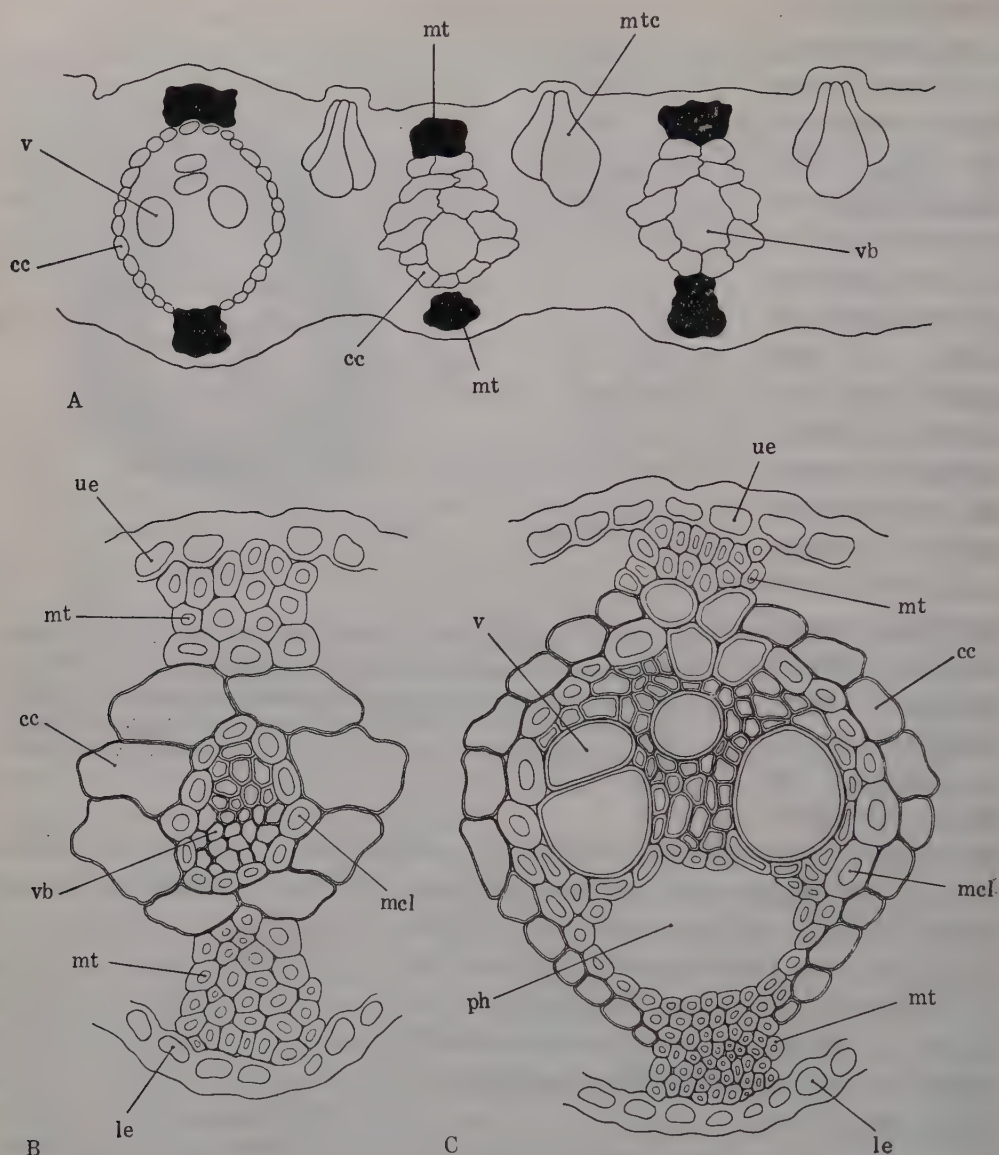
Fig. 3. Transverse leaf section of *Thysanolaena maxima* O. K. A, ×ca. 130. B, ×500, small vascular bundle. C, ×400, large vascular bundle. cc-characteristic cells which do not contain chloroplasts. le-lower epidermis. mcl-mechanical cell layer. mt-mechanical tissue. mtc-motor cells. ph-phloem. ue-upper epidermis. v-vessel. vb-vascular bundle.

Thysanolaeneae, containing only one genus, *Thysanolaena*. The spikelet structure of *Thysanolaena* is similar to that of Tristegnineae and Paniceae: spikelets two-flowered, the lower reduced floret and the upper fertile one. However, the rachilla extended beyond the upper floret and sometimes bearing a rudimentary floret is a special feature of *Thysanolaena*. Hairy 3-nerved lemma, the habit of tall robust

perennials, etc. suggest a relationship to Arundineae. Chromosemes and leaf structure in *Thysanolaena* have never been examined. The author observed the characteristics of epidermis and transverse leaf section of *T. maxima*. The results obtained are as follows:

Epidermis:—Siliceous cells are dumbbell shaped in both the lower and the upper epidermis (Fig. 2, B). Cylindric bicellular hairs are found on the lower epidermis (Fig. 2, A).

Transverse section:—Characteristic cells which do not contain chloroplasts are found surrounding the vascular bundles. Generally, they are large in small vascular bundles (Fig. 3, B, cc), and are small in large vascular bundles (Fig. 3, C, cc). Inside this cell layer, a mechanical cell layer is found (Fig. 3, B.C. mcl). Motor cells are well developed (Fig. 3, A, mtc). Chloroplasts are uniformly distributed throughout the mesophyll, and parenchymatous cells which do not arrange radially have many cell wall processes. Large mesophyll cells shared by *Streptochaeta*, *Pharus*, *Pariana*, most bamboos, etc. [cf. Page (1947)] are not found.

The leaf structure of *T. maxima* described above is very unlike that of most grasses. It strongly resembles that of Arundineae leaves. Cylindric bicellular hair, dumbbell shaped siliceous cells, the existence of cell wall processes in parenchyma, vascular bundles surrounded by a characteristic cell layer which does not contain chloroplasts, chloroplasts distributed throughout the mesophyll, etc. are features common to the two groups. C. E. Hubbard's (1934) opinion that *Thysanolaena* should be treated as an independent tribe is strongly supported by the peculiarities of leaf structure as well as by external morphology and those characteristics indicate that this tribe might be related to Arundineae.

I wish to express my cordial thanks to Dr. J. Ohwi and Dr. Y. Takenaka who gave me various valuable advices and assistance throughout this investigation.

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Two New Species of *Porphyra* and their Subgeneric Relationship[*]

by Hideo MIKAMI[**]

三上日出夫[**]： 紅藻アマノリ属の二新種とその類縁について[*]

A vegetative cell of the genus *Porphyra* is well known to have usually a single central stellate chromatophore with one pyrenoid at the center. In 1935, however, Prof. Tokida noted that the vegetative cells of *P. Onoi* Ueda, in the monostromatic part of the frond, contained two eccentric chromatophores. Further, on this interesting fact, he reported, "*P. Onoi* represents an intermediate form between the two subgenera, *Euporphyra* and *Diploderma*, not only in having a monostromatic frond intermixed with distromatic portions but also in the structure of the chromatophore." For that reason he established a new subgenus *Diplastidia* on the basis of *P. Onoi*, and it was defined as follows:

Subgenus *Diplastidia* Tokida 1935

Frond is for the most part composed of one layer of cells containing two plastids, and in portions it is often distromatic.

Now, the cells of the following two new species, which were collected by the writer in the Hidaka Prov., Hokkaido, each contained two chromatophores as *P. Onoi*. Their diagnoses and anatomical descriptions are given here as follows:

The diagnoses of the species

Porphyra pseudocrassa Yamada et Mikami

Frons membranacea, monostromatica, orbiculata vel reniformis, marginibus vix undulatis, ad basin stipitata, cordata vel rotunda, 3–7 cm. longa, 2.5–7.0 cm. lata, 55–82μ crassa; cellulis vegetativis rotundato–angulatis, in sectione transversa rotundato–subrectangularibus; gelatina superficialis 10–14μ crassa; cellulis 2–chromatophoris, substellatis (vel discoideis); dioica vel raro monoica; spermatia in antheridio 128 $\left(\dfrac{a}{4}, \dfrac{b}{4}, \dfrac{c}{8} \right)$, carposporae in sporocarpio 16 $\left(\dfrac{a}{2}, \dfrac{b}{2}, \dfrac{c}{4} \right)$.

Japanese name: Makure-amanori (nov.).

Loc.: Harutachi, Samani, Hidaka Prov., Hokkaido; Akkeshi, Kushiro Prov., Hokkaido. (The type specimen is deposited in the Herbarium of the Faculty of Science, Hokkaido University.)

Frond membranaceous, monostromatic, orbiculate or reniform, scarcely undulate at margin, shortly stipitate, with cordate or ovate base, 3–7 cm. long, 2.5–7 cm. broad, 55–82μ thick; vegetative cells, in surface view, angular with rounded corners, somewhat regularly arranged, in cross section subrectangular with round angles, usually twice as long as broad; surface jelly 10–14μ thick; lowermost cells projecting rhizoidal
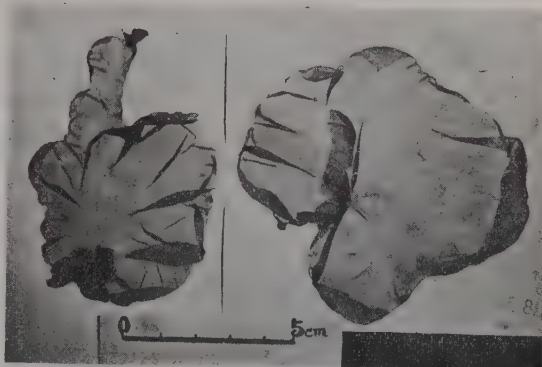

Fig. 1. *Porphyra pseudocrassa* Yamada et Mikami.

filaments ovate or capitate; each cell containing two substellate (or discoidal) chromatophores; colour of the frond purplish red or russet; dioecious or rarely monoecious; antheridia formed within yellowish marginal zone of the upper part of the frond, containing 128 antherozoids after having divided according to the formula $128 \left(\dfrac{a}{4}, \dfrac{b}{4}, \dfrac{c}{8} \right)$; each sporocarp containing 16 carpospores, after having divided according to the formula, $16 \left(\dfrac{a}{2}, \dfrac{b}{2}, \dfrac{c}{4} \right)$.

The present species grows on rocks in the upper sublittoral zone. The frond is as a rule orbiculate or reniform with scarcely undulate margin, and always monostromatic. In outer appearance, the present new species shows a considerable resemblance to *P. crassa* Ueda, described and figured by Prof. Ueda (1932) and Prof. Tanaka (1952). But it differs from the latter in the following anatomical characters. That is, according to Prof. Tanaka, in *P. crassa* Ueda each cell contains a star-shaped chromatophore with several slender arms radiating in all directions (1952, p. 56, Fig. 26,b), while this species contains two substellate (or discoidal) chromatophores. This peculiar feature is also ascertained in the basal cells which produce rhizoidal filaments, as shown in Fig. 2, 1–2. In the structure of the chromatophore, the present materials agree rather with *P. Onoi*, but differ entirely from it in being generally dioecious, in the divisional mode of antheridia and sporocarps, and in having the monostromatic thallus. On the other hand, present species shows some resemblance to some Californian species, *Porphyra lanceolata* (Setch. et Hus) G. M. Smith and *P. pulchra* Hollenberg in the structure of the chromatophore and in having the monostromatic thallus. But it is distinguishable from Smith's species by the difference of divisional mode of sporocarps outer appearances, and

from Hollenberg's species by outer appearances and its dioecious thallus. As far as the present writer's observations show, this species is dioecious, but rarely mono-ecious. Its sporocarps and antheridia are both found along the marginal turn-up portion of the frond.
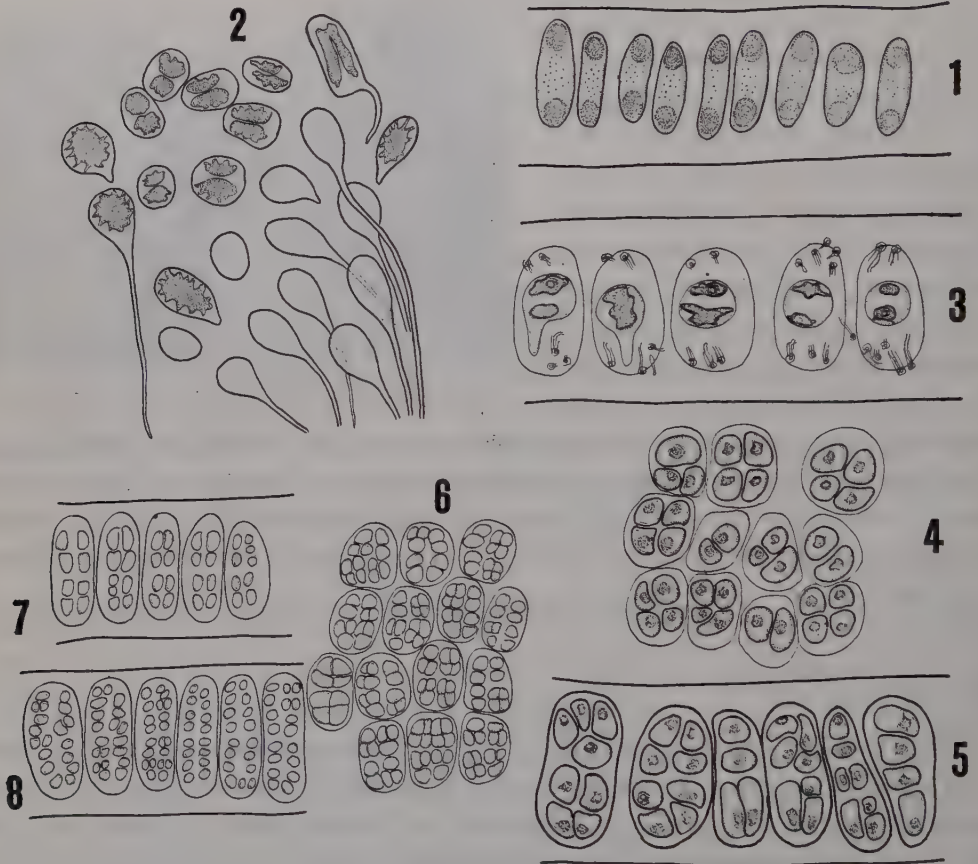

Fig. 2. *Porphyra pseudocrassa* Yamada et Mikami.

1: Section of vegetative portion, with two chromatophores in each cell.
2: Surface view of lower cells with rhizoidal filaments (dried).
3: Section of lower cells with rhizoidal filaments.
4: Surface view of cystocarpic portion.
5: Section of cystocarpic portion.
6: Surface view of antheridial portion.
7-8: Section of antheridial portion.

2, ×32.5.　　　　1, 3-8, ×370.

Porphyra punctata Yamada et Mikami

Frons membranacea, monostromatica vel distromatica, ovata vel oblongo-ovata vel lineali-lanceolata, marginibus undulatis, foraminibus rotundatis majoribus et minoribus perforata, ad basin stipitata, cordata vel rotunda, 8.5-18 cm. longa, 3.5-7 cm. lata, 50-70μ crassa; cellulis vegetativis obscure seriatis, rotundato-angulatis

vel ellipticis; gelatina superficialis 11.5–17.5 μ crassa; cellulis 1–2 chromatophoris stellatis; dioica; spermatia in antheridio $128\left(\dfrac{a}{4}, \dfrac{b}{4}, \dfrac{c}{8}\right)$, carposporae in sporo-carpio $64\left(\dfrac{a}{4}, \dfrac{b}{4}, \dfrac{c}{4}\right)$.

Japanese name: Sunago-amanori (nov.)

Loc.: Erimo, Samani, Hidaka Prov., Hokkaido. (The type specimen is deposited in the Herbarium of the Faculty of Science, Hokkaido University.)

Frond membranaceous, monostromatic or di-stromatic, ovate or oblong-ovate often linear, with undulate margins, perforate, shortly stipitate, with cordate or ovate base, lustrous, 8.5–18 cm. long, 3.5–7 cm. broad, 50–70μ thick; vegetative cells in sur-face view usually round or elliptic, ca. 17μ in dia-meter, more or less irregularly arranged; surface jelly 11.5–17.5μ thick; vegetative cells containing one or two stellate chromatophores; dioecious; sporocarpic and antheridial patches found on the mar-ginal region of the different thalli; each antheridium containing 128 antherozoids, arranged in four tiers of four each in surface view, according to the formula of division, corresponding to $128\left(\dfrac{a}{4}, \dfrac{b}{4}, \dfrac{c}{8}\right)$; sporocarp containing 64 carpospores each after having divided according to the formula, $64\left(\dfrac{a}{4}, \dfrac{b}{4}, \dfrac{c}{4}\right)$; colour of the frond purplish red.

Fig. 3. *Porphyra punctata* Yama-da et Mikami Male and female plants.

The present new species was found on rocks in the littoral zones. The shape and size of the frond are variable. The materials examined contain two forms. The one is ovate or oblong-ovate. The other has a linear frond. The fronds of the present species are for the most part monostromatic, but usually exhibit a di-stromatic nature in many portions of the full-grown frond. One of the leading characters of this species is that the distromatic parts can be detected by the naked eye as sandy spots. In some respect, *P. punctata* is similar to *P. Onoi* Ueda. As noted above, however, in the present species, some cells in the thallus contain a single typical chromatophore as shown in Fig. 4, 2, 8. In other cells in the grown portion, the choromatophore soon divides into two which gradually separate from each other. Moreover, the present species differs from it in the divisional modes of sporocarp and antheridia. The present species is dioecious. The antheridia are formed within narrow marginal zones of the frond. Each atheridium contains 128 antherozoids, arranged in four each in surface view. The formula of these division

corresponds to $128 \left(\dfrac{a}{4}, \dfrac{b}{4}, \dfrac{c}{8} \right)$. The sporocarpic patches are found in the marginal region of the different thallus. Vegetative cells are often intermixed among the sporocarps. At the matured female region the short sword-cut like streaks are often observed. As far as the writer's observations show, the matured sporocarps contain 64 carpospores. The formula of these division corresponds to $64 \left(\dfrac{a}{4}, \dfrac{b}{4}, \dfrac{c}{4} \right)$.
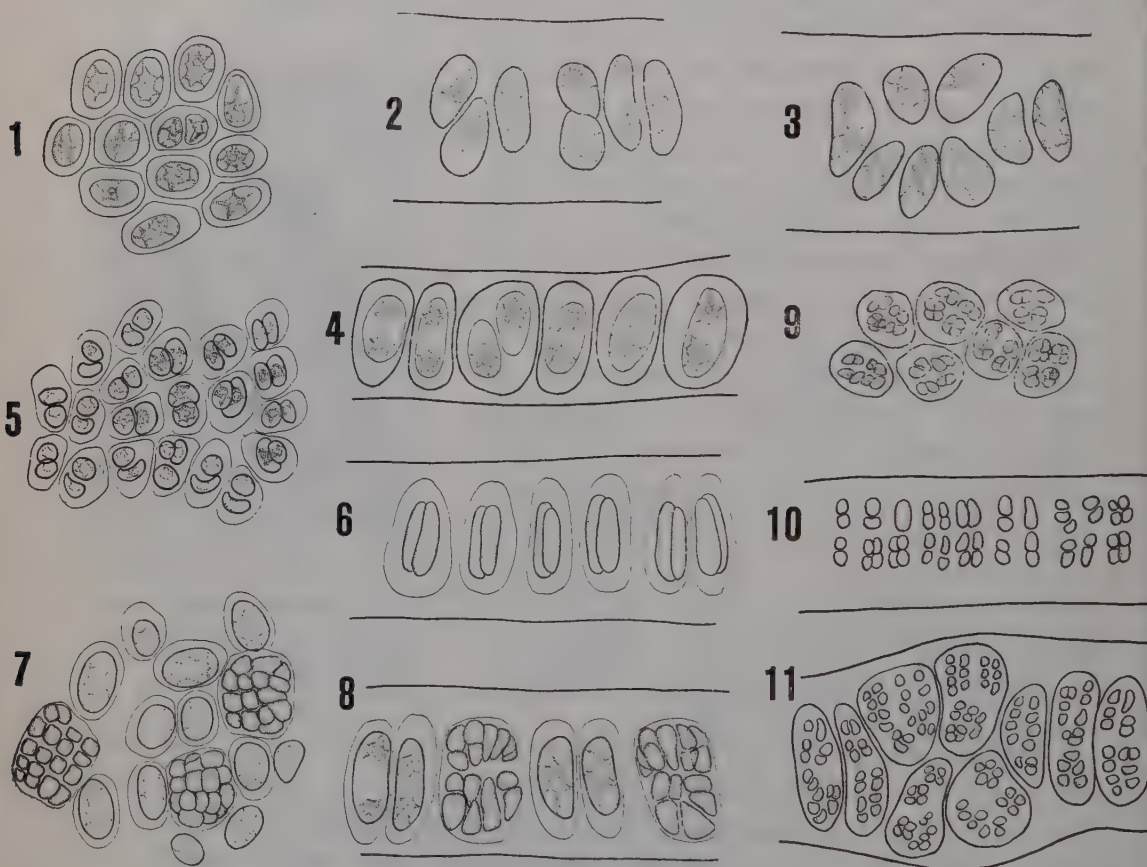

Fig. 4. *Porphyra punctata* Yamada et Mikami.

1 : Surface view of vegetative portion.
2–4 : Section of vegetative portion.
5 : Surface view of young cystocarpic portion.
6 : Section of the same portion.
7 : Surface view of mature cystocarpic portion.
8 : Section of the same portion.
9 : Surface view of antheridial portion.
10–11 : Section of antheridial portion. ×370.

Subgeneric relationship

When the subgenus *Diplastidia* was established (1935), there was known only one species, *P. Onoi* Ueda. As noted above, however, *P. Onoi*, *P. pseudocrassa*, *P. pulchra*, *P. lanceolata*, *P. punctata*, etc. resemble each other in the possession of the cells containing two plastids. As pointed out by Prof. Tokida, this character

seems to the writer to be that of an intermediate form between two subgenera, *Euporphyra* and *Diploderma*.

Judging from the resemblance in their habit, they are to be placed under the subgenus *Diplastidia*. Therefore, I propose that it is necessary to expand the definition of *Diplastidia* as follows:

Diplastidia

(1) Frond is only monostromatic, or in portions it is often distromatic.

(2) Each cell contains two plastids, or in portions a single plastid.

In conclusion, the writer wishes to tender his best thanks to Prof. Y. Yamada for his kind direction during this work.

Resume

(1) In this paper is reported the discovery of two new species of *Porphyra*, *P. pseudocrassa*, and *P. punctata*.

(2) The definition of *Diplastidia* (subg.) is expanded.

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Cytotaxonomical Notes on the *Ranunculus acris*
Group in Japan

by Hiroshi HARA* and Sachiko KUROSAWA*

原　　寛* ・ 黒沢幸子*:　日本産キンポウゲ近似種の細胞分類学的所見

The *Ranunculus acris* group is very variable in Japan as well as in continental Eurasia. From outer morphological characters, the senior author[4] has recognized 3 species with a few varieties from Japan in this group, excluding *R. yakushimensis* Masamune.

Among them, *R. japonicus* Thunberg is the commonest race in Japan widely

* Botanical Institute, Faculty of Science, University of Tokyo, Hongo, Tokyo.

distributed on plains and hills from the southwestern end of Hokkaido (Yezo) south-
wards to Ryukyu. It is readily distinguished from all other races by its sessile
stigma (Fig. 1 A), and its beakless achenes. Its petioles, leaves, and the lower part
of stems are usually densely pubescent with spreading hairs, and its basal leaves
are often incompletely divided into short-serrated broad lobes. Its nectary scale
at the base of petals is broad and almost flabellate.

The type specimens α and β of *R. japonicus* from Japan in the Thunberg
Herbarium at Uppsala surely belong to this race, but those γ and δ collected from
Hort. Upsaliensis are probably not of the Japanese origin, having a strongly hooked
beak of achenes as in *R. lanuginosus*. and should be excluded from the type of
R. japonicus.

R. japonicus is pretty variable especially in the shape of leaf-lobes, and in the
hairiness. The type of *R. Buergeri* Miquel in Herb. Leiden is a form of *R. japonicus*
with lower leaves which are shortly cut into 3 very broad depressed-roundish lobes
and only shortly serrated on the margin. Also a glabrescent form occurs here and
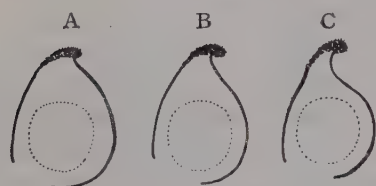

Fig. 1. Pistils of *R. japonicus*
(A), *R. grandis* var. *mirissimus* (B),
and *R. acris* var. *nipponicus* (C).

there especially on mountains of Japan, and a
similar one prevails on meadows in north China,
Manchuria, and Korea. It is often a more slender
plant with smaller leaves, and its stems and
petioles are sparsely hairy with spreading or ap-
pressed hairs or sometimes nearly glabrous. *R.
japonicus* has often been confounded with *R.
propinquus* C. A. Meyer in various literatures, but
the typical form of the latter from Altai has distinct hooked beaks, and it is ap-
pressed hairy, and is much nearer to an alpine form of *R. acris* than to *R. japonicus*.

R. yakushimensis (Makino) Masamune endemic on high mountains of Is. Yaku-
shima is certainly closely allied to *R. japonicus*. It is, however, much smaller in
all respects; and its flowering stems are low and always decumbent and radicant
at the lower nodes; and its leaves are thick, carnose, and dark green and have lobes
with a few coarse teeth.

R. acris L. var. *nipponicus* Hara[2] occurs only in the alpine zone of Hokkaido and
northern and central Honshu. Among the Japanese races, it is nearest to the typical
R. acris of Europe in the hairiness, the shape of basal leaves, and the shape of
achene-beak. Its stems and petioles are glabrescent or sparsely appressed hairy. Its
leaves are less lobed than those of var. *multifidus* DC., but its cauline leaves are
more developed than those of *R. acris* and often similar to basal leaves. *R. novus*
Léveillé et Vaniot (1906) from Japan was named to this race, and *R. subcorymbosus*
Komarov from Kamtchatka seems to be a glabrescent form of this race. This
Japanese race has formerly been referred to *R. Steveni* Andr., but true *R. Steveni*, of
course, does not grow in East Asia.

R. grandis and its varieties[3] are very characteristic in sending out long slender

subterranean stolons which have never been found in any European race. *R. grandis* var. *austrokurilensis* (Tatewaki) Hara (*R. transochotensis* Hara) is growing on grassy lowlands of Hokkaido, Kuriles, Saghalien, and Aleutians (Is. Adak), but other three races, i. e. *R. grandis* Honda, var. *ozensis* Hara, and var. *mirissimus* Hara (*R. mirissimus* Hisauchi), are very local in northern and central Honshu (Fig. 2). In general these races have somewhat interemediate characters between *R. acris* and *R. japonicus* in the hairiness, the shape of leaves, and the shape of achenes, but they differ slightly from one another in those variable characters.

The *Ranunculus acris* group has been cytologically studied by many authors. In the present investigation, we have examined chromosomes in root-tip cells of materials representing all the Japanese races mentioned above. Collected

Fig. 2. Geographical distribution of *R. grandis* and its varieties in Japan.

from the natural habitats, they have been all grown in the University of Tokyo. For counting of somatic chromosomes, the Tjio and Levan's method was used with a slight modification. The following list of chromosome numbers in the *R. acris* group was compiled from the literature, and from the authors' work.

The basic chromosome number in the *R. acris* group is 7 which has, however, been considered to be aneuploid derived from an 8-chromosome ancester of the genus and such numbers as 8, 16, and 32 were occasionally reported in this group too. The group is highly variable in the chromosome numbers, and both aneuploidy and polyploidy have been known as shown in the list. Especially aneuploidy was reported in a gynodimorphic race by Sorokin, but no aneuploidy nor gynodimorphism has been observed in Japan.

Of the Japanese races of the group, *R. acris* var. *nipponicus*, *R. japonicus*, and *R. yakushimensis* have hitherto been studied cytologically by Japanese botanists, and they were reported to have 2n=14 chromosomes. The authors also reexamined them as well as *R. acris* raised from seeds collected at Stockholm, and its var. *pumilus* collected on Mt. Nuolja in Lappland by the senior author in 1954. They proved to be all diploid (2n=14) as shown in Fig. 3, A & B. The karyotype of *R. japonicus* and *R. acris* var. *nipponicus* can be represented by the following formulae respectively.
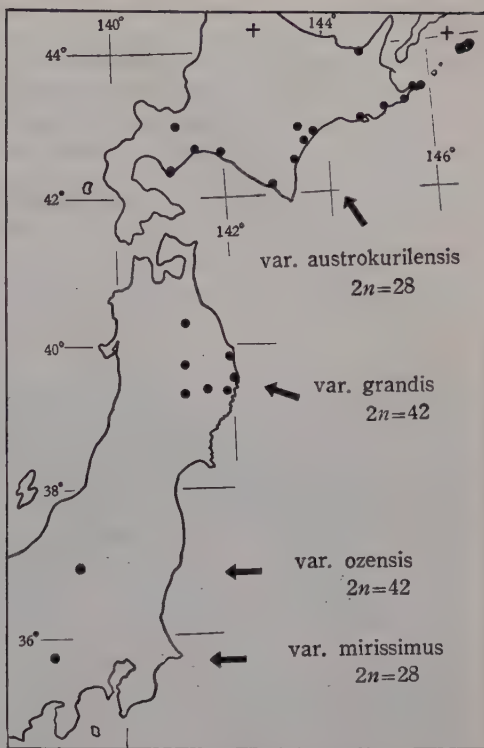
Name	2n	Locality	Author*
Rununculus acris L.	8	Austria ?	Mattick 1948
"	12	Russia (near Leningrad)	Sorokin 1924
"	13, 15, 16, 17, 18	Russia (near Leningrad) (gynodimorphic races)	Sorokin 1924 & 27
"	14	Russia (Moscow)	Senjaninova 1926
"	14	Sweden? (♀ & plen. fl. races)	Langlet 1927
"	14	U. S. A. (New York)	Sorokin 1927
"	14	England (incl. ♀ fl. race)	Whyte 1929
"	14	Sweden (Lappland; Jämtland; Stockholm); Denmark; Italy (Turin)	Langlet 1932
"	14	England (9 wild races)	Larter 1932
"	14	Denmark; Iceland	Böcher 1938
"	14	Cult. (plen fl. race)	Coonen 1939
"	14	Loc.?	Gregory 1941
"	14	Swiss	Hess 1953
"	14	Sweden (Stockholm)	Hara & Kurosawa, unpubl.
"	16	Austria (Tirol)	Mattick 1950
" var.	28	Germany (Frankfurt a. M.)	Langlet 1932
"	29–32	Russia (Moscow)	Senjaninova 1926
ssp. *Boraeanus* R. & F.	14	(Seeds from Nantes)	Langlet 1932
"	16	France	Hocquette 1922
"	28, 56	Scandinavia ?	Nygren 1948
var. *pumilus* Wahl.	14	Faroes (near Klaksvig)	Böcher 1938
"	14	Sweden (Lappland)	Löve & Löve 1944
var. *nipponicus* Hara	14	Japan	Miyaji 1927
"	14	Hokkaido ?	Sakai 1935
"	14	Honshu (Mt. Hakkoda)	Matsuura & Suto 1935
"	14	Honshu (Mt. Hakkoda; Hachimantai)	Hara & Kurosawa, unpubl.
ssp. *Friesianus* R. & F.	14	France (Nancy)	Langlet 1932
ssp. *strigulosus* Hyl.			
(*R. Steveni* Andrz. ?)	14	Denmark (cult.?)	Langlet 1927
"	14	Swiss	Hess 1953
R. acris × *R. Steveni*	21	Swiss	Hess 1953
R. japonicus Thunb.	14	Honshu	Midzuno (ex Hara) 1952
"	14	Shikoku (Matsuyama)	Kurita 1952 & 55
"	14	Honshu (Kôbe)	Hujiwara & Kondo 1955
"	14	Honshu (Tokyo; prov. Chiba; prov. Aki; prov. Shinano (a glabrescent form)	Hara & Kurosawa, unpubl.
R. yakushimensis Masam.	14	Yakushima	Kurita 1955
"	14	" ? (cult.)	Hara & Kurosowa, unpubl.
R. sp.	28	Saghalien	Matsuura & Suto 1935

* We have checked all the literatures mentioned below, but omitted to cite them in full, as they can be mostly traced from Gregory (1941) and Tischler (1950).

R. grandis Honda var. *austrokurilensis* Hara	28	Hokkaido (Nemuro; Tokachi)	Hara & Kurosawa, unpubl.
var. *mirissimus* Hara	28	central Honshu (Mitsutôge)	Midzuno (1952), unpubl.
″	28	″ (″)	Hara & Kurosawa, unpubl.
var. *grandis*	42	n. Honshu (near Morioka)	Hara & Kurosawa, unpubl.
var. *ozensis* Hara	42	central Honshu (Ozegahara)	Hara & Kurosawa, unpubl.

$$K \ (2n)=14=2A_1{}^m+2A_2{}^m+2B^{sm}+4C^{st}+2D_1{}^{st}+2^{(t)}D_2{}^{st}$$
$$K \ (2n)=14=2A_1{}^m+2A_2{}^m+2B_1{}^{sm}+2B_2{}^{sm}+4C^{st}+2^{(t)}D^{st}$$

Stoloniferous *R. grandis* with 3 varieties were here cytologically studied for the first time with certainty, although the plant of Saghalien with 14 haploid chromosomes reported by Matsuura & Suto in 1935 may belong to this race. In 1952, by the courtesy of Dr. T. Midzuno, *R. grandis* var. *mirissimus* from Mitsutôge was determined to have 2n=28 chromosomes, but the result has not been published. Now we noticed that both *R. grandis* var. *austrokurilensis* from Tokachi and Nemuro in Hokkaido, and *R. grandis* var. *mirissimus* from Mt. Mitsutôge (ca. 1600–1700 m), Prov. Kai in central Honshu are tetraploid (Fig. 4). Whereas *R. grandis* from Prov. Rikuchu in north Honshu, and *R. grandis* var. *ozensis* from Ozegahara (ca. 1400 m high) in central
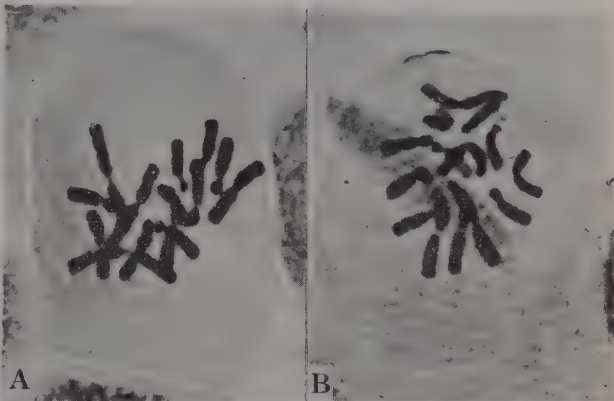

Fig. 3. Somatic chromosomes of *R. japonicus* from Prov. Aki (A), and *R. acris* var. *nipponicus* from Mt. Hakkoda (B). ×ca. 2000.
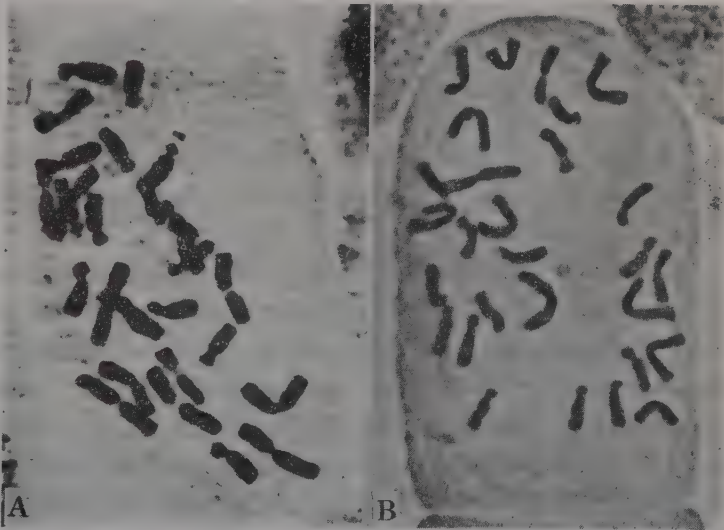

Fig. 4. Somatic chromosomes of *R. grandis* var. *austrokurilensis* from Tokachi (A), and var. *mirissimus* from Mitsutôge (B). ×ca. 2000.
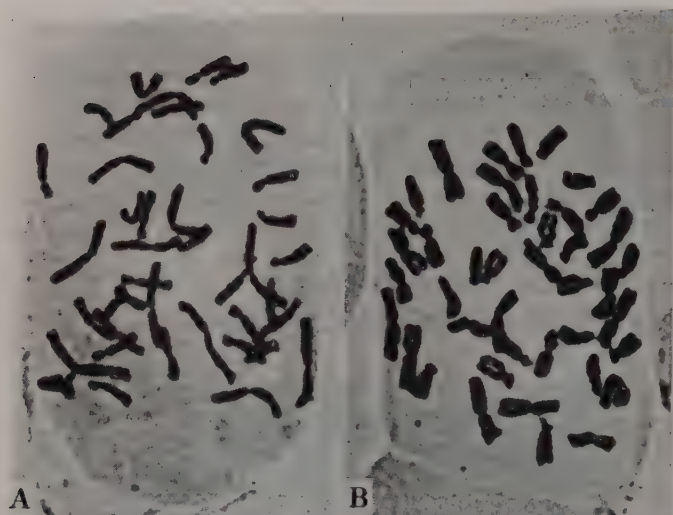
Honshu are hexaploid (2n=42) which is a new number in the *R. acris* group (Fig. 5), although hexaploid cells have very rarely been found in the tissue of the young fruits by Böcher in 1938.

The karyotypes (Fig. 6) of these races analysed from the figures 4 and 5 can be shown as follows:

Fig. 5. Somatic chromosomes of *R. grandis* var. *grandis* from near Morioka (A), and var. *ozensis* from Ozegahara (B). ×ca. 2000.

R. grandis var. *mirissimus* (*R. mirissimus*)

$$K(2n)=28=4A_1{}^m+4A_2{}^m+4B^{sm}+8C^{st}+4D_1{}^{st}+4D_2{}^{st}$$

R. grandis var. *austrokurilensis* (*R. transochotensis*)

$$K(2n)=28=4A_1{}^m+4A_2{}^m+4B^{sm}+8C^{st}+4D_1{}^{st}+4D_2{}^{st}$$

R. grandis var. *grandis*

$$K(2n)=42=12A^m+6B^{sm}+12C^{st}+12D^{st}$$

R. grandis var. *ozensis*

$$K(2n)=42=12A^m+6B^{sm}+12C^{st}+12D^{st}$$

Taking into consideration the instability of chromosome number in the *R. acris* group, and also the chromosome morphology mentioned above, an allopolyploid origin might be suggested for these polyploids.

The pollen grains of Japanese races were observed by Miss Masa Ikuse, but the distinction in shape and size among them was not clear.

The stomata on the lower surface of basal leaves were also examined. All diploid races have small and regular stomata. In *R. japonicus*, stomata are numerous and 50–63 (–72) μ long; in *R. acris* of Europe they are 49–55 μ long; in *R. acris* var. *nipponicus* 53–60 μ long, but much fewer than those of *R. japonicus;* and *R. yakushimensis* they are 53–59μ long often mixed with somewhat shrunken ones. Stomata of tetraploid *R. grandis* var. *austrokurilensis* are also regular, and 51—63μ long. In tetraploid *R. grandis* var. *mirissimus*, however, stomata are much fewer and larger than those of *R. japonicus*, and (59-) 63—88 (-93) μ long. Hexaploid

R. grandis has regular stomata (54–) 59—64
(–69) μ long, while in the other hexaploid,
R. grandis var. *ozensis*, they are irregular
in size varying from (44–) 59 μ to 88 (–98) μ
long, and chloroplasts in the guard cells are
smaller and inconspicuous as compared with
other races.

It is very interesting to consider the
phylogenetic interrelationships between the
Japanese races investigated here. And it
is evident that polyploidy played an impor-
tant rôle in the evolution and speciation
of this group. Before the problem can be
fully discussed, we should of course study
more ample material from continental Asia,
and further cytogenetical studies including
cross experiments between various races are
much needed. However, judging from the
data now at hand, the following assumption
regarding the history of evolution in the
R. acris group of East Asia may be admis-
sible.

Until the middle Tertiary, the ancestor
of the *R. acris* group may not have reached
East Asia. The fact that the group is not
native in North America supports this view.
First of all, *R. japonicus* more fitted to warmer
temperate climate has differentiated from
the ancestor in the diploid level, and has
gradually spread eastwards over China and
Japan. Then a tetraploid race (possibly *R.
grandis* var. *austrokurilensis*) which acquires
a stoloniferous habit has arisen from *R.
japonicus* or from its progenitor, and has
been distributed northwards.

Fig. 6. Somatic chromosomes of (1)
Ranunculus japonicus, (2) *R. acris* var.
nipponicus, (3) *R. grandis* var. *mirissi-
mus*, (4) var. *austrokurilensis*, (5) var.
grandis, and (6) var. *ozensis*. × ca. 1500.

Later during the Ice Age, *R. acris* has extended its area widely over Eurasia.
A tetraploid race (*R. grandis* var. *austrokurilensis*) has spread southwards at least
to the middle part of Honshu which had once been occupied by *R. japonicus*. And
during the maximum or the last glaciation, *R. acris* has finally penetrated through
Hokkaido into the central part of Honshu together with many other widespread
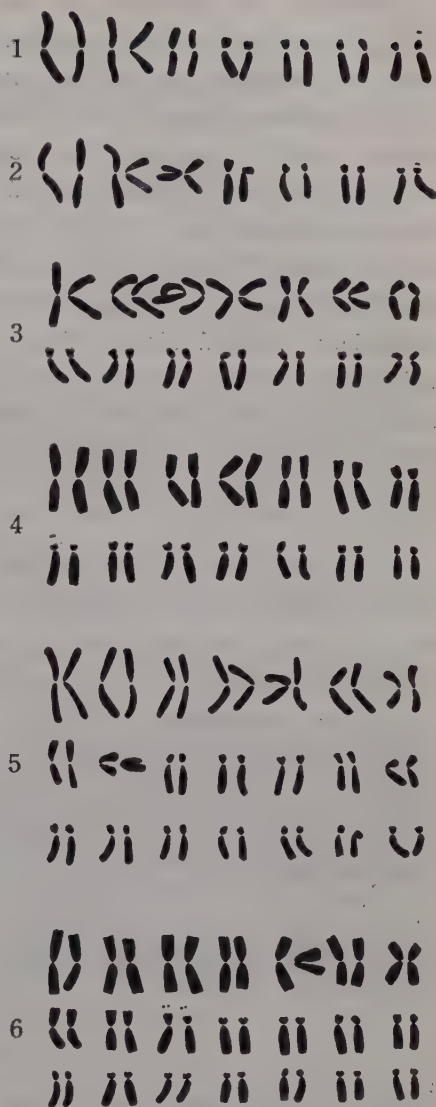boreal plants which are now found in the alpine region of central Honshu.

After the last glaciation, *R. acris* which had once occupied a wide continuous area in north and middle Japan has survived only in the alpine zone on high mountains, and was now isolated. *R. grandis* var. *austrokurilensis* which might have also had a continuous range is now almost perished in middle Honshu. *R. grandis* var. *ozensis* might be considered as hexaploid derived from var. *austrokurilensis* and has persisted only in woods around the Ozegahara moor where many other boreal plants are found quite isolated from their main areas. *R. grandis* of northern Honshu (also hexaploid) and var. *mirissimus* of central Honshu (tetraploid) are other allied races with long subterranean stolons. It is still doubtful whether they have been derived directly from var. *austrokurilensis*, or they, especially var. *mirissimus*, can be assumed to originate from *R. japonicus* independent from *R. grandis* var. *austrokurilensis*. It may be probable that these polyploid races now found isolated in Honshu are of polyphyletic origin.

Summary

All seven races of the *Ranunculus acris* group growing wild in Japan were cytologically examined. Besides three diploid races which have hitherto been reported, two tetraploid races (2n=28) and two hexaploid races (2n=42) were here recorded for the first time. It is noteworthy that all these four polyploid races have a striking characteristic of having long subterranean stolons, and occupy definite geographical areas, and therefore, they have been treated as *R. grandis* Honda in a wide sense by the senior author, notwithstanding the variability in other outer morphological characters. Probable interrelationships between those races were also discussed.

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Flowering Responses in *Pharbitis Nil* in Relation to the Leaf Area Subjected to Inductive Photoperiod

Shun-ichirô IMAMURA* and Atsushi TAKIMOTO*

今村駿一郎,* 滝本　敦*: アサガオの日長感応に及ぼす葉面積の影響

I. Introduction. It has been demonstrated in many plants that the leaves are the locus of photoperiodic induction. In a developing axillary bud of *Xanthium*, a leaf area of 0.2 cm² in total, when subjected to a single short day, can induce flowering (6). Dark treatment of a portion of a leaf is also effective in some plants (1, 2, 3). In the present studies attempts were made to obtain some information about the quantitative relationship between the dimension of the leaf area and photoperiodic reaction.

Japanese morning glory, *Pharbitis Nil*, strain " Violet ", was used as in previous experiments (4).

As methods, plants grown under continuous illumination were decapitated above the second node, whose leaf was used as donor and its axillary bud as receptor. All lower nodes were deprived of their leaves and buds. The leaf area at the second node was reduced to a desired size by removing an adequate piece of the blade. After being exposed to a given short day treatment, the plants were returned to the bench illuminated by natural day light supplemented with artificial light at night. The observation was made after about 2 weeks, when the receptor bud had developed to a considerable size.

II. Experimental results.

Experiment 1. Twenty plants were transplanted in 4 rows of 5 plants in each. At the beginning of the experiment, plants were selected for uniformity and inferior plants were removed. The plants were decapitated above the third node. In the control lot the whole leaf blade was removed. In two other lots the leaf blade was restricted to a very small and a somewhat larger piece, respectively, above the petiole by cutting off proportionate parts. In the last lot the whole leaf remained intact. The leaf areas measured by a planimeter at the end of the experiment were 2.8, 10.4 and 39.6 cm² on the average, respectively. To make the plants and the prevailing conditions in all experimental lots as uniform as possible, experiments

* Laboratory of Applied Botany, Faculty of Agriculture, Kyoto University.
京都大学農学部　応用植物学研究室

were designed so that each of the 4 rows contained in one box had different leaf areas. They were given five short days consisting of an 8 hour light period and a 16 hour dark period, thereafter they were transferred to continuous illumination. As shown in Table 1, the plants deprived of a whole leaf blade remained vegetative

Table 1. Photoperiodic induction in relation to the leaf area exposed to 5 short days

(Sown on June 10, experiment started on July 13, 1950).

Average leaf area exposed to dark period in cm^2	0	2.8	10.4	39.6
No. of plants with flowers / No. of plants observed	0/20	19/20	17/17	20/20
No. of flower buds	0	75	102	181
Average number of flowers per plant	0	3.8±0.40	6.0±0.33	9.1±0.31
No. of plants with terminal flower	0	0	1	10
Average number of the node bearing the 1st flower primordium	—	2.6±0.12	3.1±0.12	2.3±0.13

without initiating a single flower primordium. Nineteen out of 20 plants with the leaf area of 2.8 cm^2, initiated 75 flower primordia in the total. All plants of the other two lots produced flower primordia. The average numbers of flowers per plant were 3.8, 6.0 and 9.1 respectively, indicating an increasing response with increasing leaf area left after the operation.

Experiment 2. In this experiment the leaf blades were restricted to 1 cm^2, 4 cm^2 and the basal half of the area. Plants with a whole leaf and without leaf blade were also used as controls. Following a 5 short day treatment they were grown under continuous illumination. The results are represented in Table 2. The plants

Table 2. Photoperiodic induction in relation to the leaf area exposed to 5 short days

(Sown on Sept. 19, experiment started on Oct. 17, 1949).

Area of the leaf exposed to dark period	0	1 cm^2	4 cm^2	basal half of the leaf	whole leaf
No. of plants with flowers / No. of plants observed	0/16	16/16	16/16	15/15	14/14
No. of flower buds	0	80	94	112	123
Average number of flowers per plant	0	5.0±0.27	5.9±0.20	7.5±0.47	8.8±0.28
No. of plants with terminal flower	0	0	0	6	7
Average number of the node bearing the 1st flower primordium	—	2.5±0.20	2.3±0.17	1.3±0.12	1.2±0.11

deprived of the entire blade did not form a single flower primordium. All other plants, even those which had only a small piece of leaf blade of 1 cm² left, initiated flower primordia. The number of flower buds increased with the increasing leaf area subjected to short day treatment. The plants with one half and one whole leaf blade initiated 6 and 7 terminal flower buds, respectively. With the reduction of the leaf area, indications are that the flower primordia appear later, as revealed by the average number of the node bearing the first flower primordium. This may be due to the fact that it takes a longer time for a small leaf area than for the whole leaf to supply an effective amount of stimulus to the receptor.

Experiment 3. In two lots the blade of the 2nd leaf was reduced to one quarter and one half of its area, respectively. In another lot the 2nd leaf and in the last lot the 1st and 2nd leaves were left intact. The average leaf area measured at the end of the experiment was 7.8 cm², 13.9 cm², 23.8 cm² and 42.7 cm² respectively. Plants deprived of all leaf blades were also used as control. The results are represented in Table 3. Plants without leaf blades did not produce flower primordia. In

Table 3. Photoperiodic induction in relation to the leaf area exposed to 5 short days

(Sown on June 5, experiment started on June 27, 1950).

Leaves exposed to dark period	Control	Basal one quarter of the 2nd leaf	Basal half of the 2nd leaf	2nd leaf	1st and 2nd leaves
Leaf area in cm²	0	7.8	13.9	23.8	42.7
No. of plants with flowers / No. of plants observed	0/20	17/19	19/19	19/20	18/18
No. of flower buds	0	62	81	89	102
Average number of flowers per plant	0	3.3±0.38	4.3±0.39	4.5±0.39	5.7±0.36
No. of plants with terminal flower	0	0	1	3	11
Average number of the node bearing the 1st flower primordium	—	1.8±0.11	1.6±0.12	1.5±0.12	1.4±0.12

the lot with one quarter of leaf area left, 17 out of 19 plants produced 62 flower primordia in the total. All plants of the remaining three lots produced flowers except one in the lot having the 2nd leaf intact. The number of flower primordia initiated increased with the increasing area of the donor leaf. Also more terminal flowers were initiated with the increase of the area. A rising tendency of the node bearing the first flower when the leaf area was decreasing is also evident.

Experiment 4. As reported in the previous paper, flower primordia can be initiated in *Pharbitis Nil* even when the leaf is entirely removed after having been exposed to a single dark period of sufficient duration. The time, in which the stimulus may

be transmitted from the leaf in an effective amount to cause flower initiation, may also vary with the area of the leaf exposed to photoperiodic treatment. This possibility was investigated in further experiments. A hundred and twenty plants were planted in 6 boxes with 20 plants in each. Each box contained 4 rows, 5 plants per row, and each row received a different reduction of the leaf area. In 3 rows the area of the second leaf was reduced to 1.0 cm^2, 4.0 cm^2 and one half of the blade respectively. The plants of the remaining row had the second leaf intact. All boxes were covered with wooden boxes. After 18 hours the covers were removed from 2 boxes and immediately the donor leaf was cut off. Other 2 boxes were darkened for 24 hours and then defoliated. The remaining 2 boxes were defoliated after a dark treatment of 30 hours' duration. Table 4 shows the results obtained. In plants

Table 4. Relation between the area of donor leaf and transmission time of the photoperiodic stimulus from the leaf. Leaf was restricted to the given area before dark treatment

(Sown on May 20, experiment started on June 18, 1952).

| Group | Duration of the dark period given before removal of the leaf, in hours | Responses | Average leaf area in cm^2 | | | |
			1.0	4.0	15.3 (basal half of the leaf)	33.1 (whole leaf)
1	18	No. of plants with flowers / No. of plants observed	0/10	0/9	1/10	4/10
		No. of flower buds	0	0	3	12
2	24	No. of plants with flowers / No. of plants observed	0/10	0/10	4/10	6/10
		No. of flower buds	0	0	10	12
3	30	No. of plants with flowers / No. of plants observed	0/10	0/10	3/10	6/10
		No. of flower buds	0	0	6	22

with leaf blades of 1 cm^2 and 4 cm^2 no flower formation occurred, even when the dark treatment lasted 30 hours. Some of the plants with one half of the blade left initiated flower primordia following dark periods of any duration. Plants whose whole blade of the 2nd leaf was left intact produced more flower primordia than the preceding lots.

Experiment 5. Three hundred and sixty plants were transplanted in 18 boxes with 20 plants in each, and divided in 3 groups consisting of 6 boxes. Three lots of each group had leaf blades of 4 cm^2, 12.6 cm^{2*} (basal half of the leaf) and 28.3 cm^{2*} (whole leaf), respectively. The rows of 5 plants, each with a different leaf area,

* Measured at the conclusion of the experiment.

were arranged in each box in randomized order. They were then transferred to the dark room and each group was defoliated after dark treatment of 18, 24 and 30 hours respectively. The results are indicated in Table 5. Three plants with leaf

Table 5. Relation between the area of donor leaf and transmission time of photoperiodic stimulus from the leaf

(Sown on May 26, experiment started on June 24, 1952).

Group	Duration of the dark period given before removal of the leaf, in hours	Responses	Average leaf area in cm²		
			4.0	12.6 (basal half of the leaf)	28.3 (whole leaf)
1	18	No. of plants with flowers / No. of plants observed	0/36	0/35	8/36
		No. of flower buds	0	0	14
2	24	No. of plants with flowers / No. of plants observed	0/35	6/35	27/36
		No. of flower buds	0	21	97
3	30	No. of plants with flowers / No. of plants observed	3/36	14/36	19/36
		No. of flower buds	4	33	65

blades of 4 cm² initiated flower primordia only when the dark treatment before defoliation lasted 30 hours. Six out of 35 plants with one half of leaf area which was exposed to 24 hour darkness developed flower primordia upon the removal of the remaining half. Of the 36 plants with whole leaf blades which were defoliated after 18 hour dark period, 8 plants were induced to initiate flower primordia. The increasing response with the lengthening of dark period before defoliation is, in spite of some irregularities, obvious.

III. Discussion. Recently Khudairi and Hamner (6) studied in detail the relative sensitivity of leaves of different age in *Xanthium* and found that a young developing leaf on the main axis having an area of 1–2 cm² cannot cause flowering, whereas in a developing cotyledonary bud a leaf area of 0.2 cm² in total is sufficient to cause response to a single dark period of 16 hours' duration. Some authors claim that defoliated stems of some short day plants may be induced to flower when given repeated inductive photoperiodic cycles (7, 8). In *Pharbitis*, none of the plants deprived of all leaf blades, at least under short day treatment of 5 days, initiated a single flower primordium in all above and other, here not mentioned experiments. In some individuals a leaf area of only 1 cm², when given 5 short days, can induce the growing point of its axillary bud to initiate flower primordia. The photoperiodic response increases with the increasing leaf area subjected to photoperiodic treatment,

but the increment in response is not proportional to the size of leaf area. Under favorable conditions small pieces of a leaf blade can produce more flower buds than expected from the dimension of the leaf area (Tables 1 and 2).

The time, in which the stimulus is transmitted from the donor leaf in effective amounts to cause flower initiation, increases with decreasing leaf area. Experiment 5 indicates that it lies between 24 and 30 hours in plants with the leaf area of 4 cm², between 18 and 24 hours in plants with one half of leaf area, and is shorter than 18 hours in plants with a whole leaf blade. The intensity of photoperiodic induction is closely related to the size of the leaf area which receives the dark treatment.

In defoliation experiments (Tables 4 and 5) some irregularities were found when the dark period before the removal of the donor leaf was prolonged. The response decreased in 30 hours' lots of Tables 4 and 5. Such cases were also encountered in another experiment previously reported (5). What circumstances were responsible for this behavior cannot be explained without a further experiment under controlled conditions, since the experiments reported here were performed in an uncontrolled greenhouse where the external conditions varied considerably.

Summary

1) Plants deprived of all leaf blades cannot be induced to initiate flowers when exposed to an 8 hour light period followed by a 16 hour dark period lasting 5 days.

2) A leaf area reduced to 1 cm² is sufficient to cause flower initiation when subjected to 5 short days.

3) Under favorable conditions some individuals whose leaf blade is restricted to 4 cm² can cause flower initiation when subjected to a 30 hour dark period followed by immediate defoliation.

4) Flowering response increases with increasing leaf area but the increment in response is not proportional to that of the leaf area. A small piece of leaf can produce relatively more flower buds than expected from its size.

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Studies on Cerophilic Growth of Moulds on Wax and Paraffin

by Morieko IMAI*

今井百里江子*： 糸状菌の蠟及びパラフィン上の発育について

Introduction

Numbers of papers[1],[2],[3],[4],[5],[6],[7] have been published describing microorganisms capable of growing on wax or paraffin, since Söhngen[1] reported for the first time his findings with Mycobacteria in 1913. In 1925 H. Molisch[8] isolated several strains of moulds growing on the surface of bamboo culm and showed that they were capable of utilizing wax as the substrate for their growth. Molisch gave the name "cerophile Pilze" to the group of moulds. Tausson[9] demonstrated that paraffin and beeswax were oxidized by one of the commoner moulds, *Aspergillus flavus*. More recently Y. Miyamoto[10],[11] reported the isolation of a mould species which decomposed wax. It seems, however, that there is much work to be done on this group of organisms. In fact, the author of the present paper has isolated several years ago one such organism belonging to the class of *Fungi Imperfecti* from a culm of bamboo.

In this paper will be reported the results of investigations on the cerophilic growth of this group of organisms, including the above mentioned strain of mould, referred to as Strain W1 in this paper, and also other forms obtained from similar sources. For the purpose of comparison, the results with commoner species of moulds will also be described.

Experiments and results

I) *Isolation of moulds from bamboo culm.*

The surface of the culm, especially in the regions near the node, is covered with a thin layer of exudated wax substance[12],[13] powdery white in appearance. As the plant grows it commonly occurred that this white patch became gradually discoloured into black, the colour change being usually initiated at the margin of the patch. Microscopic examination of such material always disclosed more or less abundant growths of mould forms, which then were transferred on artificial culture media and the organisms were isolated as follows.

* Botanical Laboratory, Faculty of Science, Ochanomizu University, Tokyo.
お茶の水女子大学理学部植物学教室

Methods for isolation. Two methods were devised for isolating the organisms in question. (i) A small piece of wax mounted on a slide-glass was warmed until it melted to make a thin layer of wax on the glass surface. The test material i. e. small portion of infected wax layer (see above) torn off from the bamboo culm with a knife was placed on the solid wax surface, which then was kept for 7 days in a moist chamber at 28°–30°C. Part of the mycelial growth stretching themselves on the wax surface was picked up to be transferred further to the synthetic culture medium (see later). (ii) As an alternative, the plate culture method was adopted. Small pieces from the wax film covering the surface of the culm were dropped into a test tube containing sterillized water, the content of the tube was smeared on the surface of an ordinary malt-agar plate, and the colonies of fungal forms appearing after 3–7 days of incubation at 28°–30°C. were transferred to the synthetic medium for further investigation. The latter method of isolation furnishes chances also for development of ordinary non-cerophilic forms, which might have originated from spores accidentally contaminating the wax surface, as disclosed by the ensuing growth tests on wax media. Through these methods of isolation, pure cultures of cerophilic moulds were obtained from various bamboo species from several habitats in the vicinity of Tokyo. These included 22 strains of moulds which the present author has identified as follows: *Alternaria* (7 strains), *Curvularia* (2 strains), *Nigrospora* (2 strains), *Penicillium* (2 strains), *Aspergillus* (1 strain) and *Fungi Imperfecti* (W1 and 7 other strains). More detailed description of these organisms will be published elsewhere.

II) *Tests for cerophilic growth.*

As the starting material wax and paraffin were used; paraffin (m. p. 55°–56°C.) Merck, and paraffin (m. p. ca. 50°C.) Junsei Co. Ltd. Japan and yellow bees wax (first class guarantee). After freeing from solid contaminants by filtrating through filter paper, the liquefied mass of wax or paraffin was washed with a large quantity of hot water under vigorous shaking in a boiling water bath. The wash-water was tested in regard to its content of substances which might be utilized as the nutrient materials in non-cerophilic growth of the moulds. To the wash-water in question, the necessary mineral nutrients (Czapek's solution) were added and the solution was inoculated with spores of *Aspergillus fumigatus* or *Penicillium notatum* and incubated at 24°–28°C. for a month. Negative results in the test was considered as indicating the absence of nutrient impurities in the preparation used. At the same time, chemical tests including colour reaction with concentrated sulfuric acid, the decolourization of potassium permanganate solution, and the colour reaction with phenolphthalein were also adopted. All the cultures in this study were carried out with preparations warranted for purity in this regard.

a) *Culture on wax surface.* Slide-glass covered with paraffin or wax (see above) was inoculated with spores from cultures of organisms to be tested. After incubating

for 50 days in a moist chamber at 29°–30°C., the growth on the wax surface was examined with naked eyes and with a microscope. The results are summarized in Table 1. Most luxuriant growth on paraffin was obtained with Strain W1 and B3,

Table 1. Growth of isolated mould strains on wax and paraffin (slide-glass culture).

	Organism	Paraffin			Beeswax		
		T°C	Days	Growth	T°C	Days	Growth
C6	*Aspergillus niger*	28	30–50	—			
C5	*Penicillium* sp.	28	30–50	—			
PX	*Penicillium* sp.	28	50	—	28–30	50	—
A1	*Nigrospora* sp.	28	30	—			
A2	*Curvularia* sp.	23	30	+			
A5	*Curvularia* sp.	28	30	±			
W2	*Alternaria* sp.	30	12–43	+	30	12–43	++
A3	*Alternaria* sp.	28	30	—			
A4	*Alternaria* sp.	28	30	—			
C1	*Alternaria* sp.	28	30–50	+			
C2	*Alternaria* sp.	28	30–50	+			
C3	*Alternaria* sp.	28	30–50	+			
C4	*Alternaria* sp.	28	30–50	±~+			
W1		28–30	12–43	+++~++++	28–30	12–43	++++
B1		28	30–50	+			
B2		28	30–50	+			
B3		28	30–50	+++			
C8		28	30–50	—			

less marked results with nine other strains. Growth was also positive with Strain W1 and another belonging to the genus *Alternaria*, both of which being paraffinovorous as stated above. Among 18 strains of moulds tested, 7 were shown to be unable to grow under the conditions of this experiment: among these were *Aspergillus niger, Penicillium* sp., *Nigrospora* sp. and three other strains of *Fungi Imperfecti.*

　　b) *Culture on liquid media.* As an alternative wax or paraffin was added to the basal inorganic medium (Czapek's solution) as the sole source of carbon and heat-sterilized in an autoclave. The mass of wax substances was stirred up vigorously before cooling as to give rise to groups of floating isolated flakes on the liquid surface when the medium is completely cooled. In another series sucrose instead was added to the basal medium. Table 2 presents the results obtained after incubating the inoculated flasks (Erlenmeyer flasks of 100–200 ml volume) for 33–54 days at 28°–30°C. The growth marked with ++++ was very vigorous, covering the whole liquid surface with hyphae; those indicated with (±) were scanty, forming thin

Table 2. Growth of isolated moulds on wax and paraffin (liquid culture).

Organism		Days	Paraffin		Beeswax		Sucrose	
			g. added	Dry wt. of mycelium (mg)	g. added	Dry wt. of myeelium (mg)	g. added	Dry wt. of mycelium (mg)
C6	*Aspergillus niger*	50	0.3	48.5	—	—	1.5	220.0
C5	*Penicillium* sp.	50	0.3	16.0	—	—	1.5	590.0
PX	*Penicillium* sp.	49	0.3	52.8	0.7	68.2	1.5	434.0
Al$_1$	*Nigrospora* sp.	50	0.7	21.0	—	—	3	576.0
A2	*Nigrospora* sp.	50	0.7	25.5	—	—	3	542.0
A1	*Curvularia* sp.	50	0.3	47.7	—	—	1.5	218.0
W2	*Alternaria* sp.	50	0.7	21.0	—	—	8	593.0
C1	*Alternaria* sp.	49	0.3	7.0	0.3	5.0	1.5	353.0
W1		50	0.7	100.4	—	—	3	1045.5
		49	0.3	64.0	0.3	94.4	1.5	1216.0
WD		49	0.3	4.0	0.3	2.4	1.5	469.0
WE		49	0.3	10.8	0.3	4.2	1.5	290.0
WJ		49	0.3	9.0	0.3	8.0	1.5	121.0

patches of mycelium scattered over the surface of the medium. Mycelial mass was collected on filter paper, rinsed with water, dehydrated by pouring a small amount of alcohol, extracted with chloroform to remove portions of paraffin or wax adhering to the harvested mass, dried and weighed. Control runs without the addition of carbon source to the basal medium always gave rise to negative results.

It is worthwhile mentioning that the yields in dry weight of mycelium were as high as 10–20% that of wax or paraffin added, there being no doubt as to the cerophilic nature of the growth observed.

In view of these observations it was thought that cerophilic growth might not be rare among the mould species, and commoner species from other sources were tested in this regard. Tables 3 and 4a present the results of slide-glass and liquid medium culture, respectively, performed as described earlier. From the results in

Table 3. Growth of common mould species on wax and paraffin (slide-glass culture).

Organism	Paraffin			Beeswax		
	T°C	Days	Growth	T°C	Days	Growth
Aspergillus oryzae	28	15–20	+~╫	28	50	+~╫
Aspergillus oryzae 383	28–30	50	±~+	28–30	50	±
Aspergillus flavus	28	35–50	+	28	35–50	±
Aspergillus glaucus var. *tonophilus*	28	50	±	28	50	+
Penicillium notatum P1	28	15–20	╫	——	——	——
Penicillium notatum 06	28–30	35–50	+	28–30	35–50	+
Monascus araneosus	28–30	21–50	+~╫	28–30	21–50	+~╫
W 1	28–30	12–43	╫~╫╫	28–30	12–43	╫╫

Table 4a.　Growth of common mould species on wax and paraffin (liquid culture).

Organism	Days	Paraffin		Beeswax		Sucrose	
		g. added	Dry wt. of mycelium (mg)	g. added	Dry wt. of mycelium (mg)	g. added	Dry wt. of mycelium (mg)
Aspergillus niger	49	0.7	49.0	0.7	67.0	1.5	479.0
Aspergillus oryzae	50	0.7	66.5	———	———	3	650.0
Aspergillus oryzae 383	54	0.7	115.0	0.7	104.0	4	645.2
Aspergillus flavus	49	0.3	39.0	0.3	57.0	1.5	585.0
Aspergillus fumigatus 5052	49	0.7	42.0	0.7	79.6	4	419.4
Aspergillus fumigatus 5442	49	0.7	82.8	0.7	100.4	4	593.4
Aspergillus fumigatus 5454	54	0.7	35.2	0.7	27.4	4	875.2
Penicillium notatum P1	54	0.7	69.8	———	———	4	829.6
Penicillium notatum 06	50	0.7	156.0	———	———	3	463.0
Monascus araneosus	49	0.7	68.6	0.3	33.6	1.5	351.6
Alternaria sp.	49	0.3	10.2	0.3	13.0	1,5	290.6
Cladosporium herbarum	49	0.3	1.0	0.3	0	1.5	279.6
W1	49	0.3	64.0	0.3	94.4	1.5	1216.0

the tables follows that the supposition was actually the case with some of the test organisms. It seems, however, that the capacity for growing on wax or on paraffin is not necessarily a feature that characterizes one mould species from another, for there are occasionally wide differences in this respect among strains belonging to the same species. The results of shake-culture are shown in Table 4b. It is apparent from these results that, cerophilic growth represents a feature rather common among mould forms.

III)　*Culture on other wax-like substances.*

　　Wax-like substances including homologues of paraffin (i.e. paraffin, vaseline,

Table 4b.　Growth of common mould species on wax and paraffin (liquid shake culture).

Organism	Days	Paraffin			Beeswax		
		Growth on paraffin pieces	Growth in liquid	End-pH	Growth on beeswax pieces	Growth in liquid	End-pH
Aspergillus niger	35	╫	—	6.2	+	±	5.8
Aspergillus oryzae 383	35	╫	—	5.8	╫	—	5.8
Aspergillus flavus	28	╫	+	6.8	╫	+	6.4
Aspergillus fumigatus 5052	35	╫	+	6.2	+	—	5.8
Penicillium notatum P1	30	+	—	5.8	+	—	8.4
Penicillium notatum 06	34	╫	+	7.4	———	———	———
Penicillum sp. PX	30	╫	╫	8.2	╫	+	8.2
Monascus araneosus	28	╫	+	6.6	╫	+	6.0
Cladosporium herbarum	35	+	—	5.8	+〜╫	—	5.8
A2	35	╫	—	5.8	+	—	5.6
W 1	30	╫	╫	8.0	╫	╫	6.2

liquid paraffin), various waxes (beeswax, lanoline, wood wax), olive oil, higher members of fatty acid series (oleic, stearic, palmitic, myristic, lauric and capric acids) together with the allied compounds such as cetyl alchohol, glycerol, cholesterol and carbowax, were tested for their effectiveness in supporting growth of mould strains under investigation. Table 5 and 6 summarize the results obtained by the

Table 5. . Growth on various waxes and allied substances (slide-glass culture).

Substrate	W1	Aspergillus oryzae	Aspergillus flavus	Aspergillus glaucus var. tonophilus[14]	Penicillium notatum 06	Penicillium sp. PX	Monascus araneosus[15]
paraffin (Merck I)	⫴	+	±~+	±~+	+	+	⫲
paraffin (Merck II)	⫼	+	±	±	+	±	+
paraffin (Junsei)	⫼	±~+	+	±	+	±	⫲~⫴
liquid paraffin	⫲	±	±	±	+	⫲	±
vaseline (white)	⫴	±	⫲	±	⫲	⫲	⫲
beeswax	⫴	±	±	±~+	+	±	⫲
wood wax	⫼	+	±~+	±	+	±	+~⫲
olive oil	⫼	+~⫲	⫴	−	+	⫲	+
cetyl alcohol	⫼	±	+·	±~+	+	±	⫴
unknown alcohol	±~+	—	—	—	—	—	—
nonacosane	⫲~⫼	—	—	—	—	—	—
slide-glass surface	+~⫲	±	±	±~+	±	+	±~+

paraffin (Merck I): m. p. 55°–56° C.
paraffin (Merck II): m. p. 42°–44° C.

slide-glass culture and of liquid medium culture, respectively. Methods in preparing culture media in these experiments were in principle the same as described above. Remarkable is the fact that our test organisms proved themselves to be almost omnivorous as far as the substances investigated in the present experiments were concerned. No essential difference discerning between the wax inhabiting form W1 and other laboratory mould strains was observed, although the results with the former were mostly superior to those with the latter organisms, especially when we refer to the slide-glass culture.

IV) Growth measurement with Strain W1 and Aspergillus flavus.

Cerophilic growth of two of the test organisms, one from wax-covered natural habitat and another from the ordinary laboratory stock, was followed over the period of seven weeks of incubation on a medium containing paraffin or beeswax as the sole source of carbon. To Erlenmeyer flasks (100 ml volume) containing 50 ml each of basal inorganic medium, 0.35 g each of paraffin or beeswax was added, pH adjusted to 5.8 with sodium hydroxide. Seven flasks in each series were inoculated simultaneously and incubated at 30 °C. One flask each of W1 and of *Aspergillus flavus* series was taken out at a time and used for the measurements. The dry

Table 6. Growth on various waxes and allied substances (liquid culture).

Substrate		W1			Aspergillus flavus		
Kind	g. added	End-pH	Dry wt. of mycelium (mg)	Growth	End-pH	Dry wt. of mycelium (mg)	Growth
none	0	6.0	34.0	±	6.2	1.2	±
sucrose	1.5	8.4	467.0	ℍℍ	6.2	379.0	ℍℍ
paraffin (Merck I)	0.35	6.0	37.0	ℍℍ	6.6	30.0	++
paraffin (Merck II)	0.35	6.2	61.4	ℍℍ	6.8	30.0	++
paraffin (Junsei)	0.35	6.4	48.2	++	6.6	30.0	++
liquid paraffin	0.35	5.8	55.0	ℍℍ	6.0	28.0	++
vaseline (white)	0.35	5.8	48.0	ℍℍ	6.0	64.0	ℍℍ
beeswax	0.35	6.8	57.0	ℍℍ	6.6	24.0	+
lanoline	0.5	6.0	31.6	++	6.0	48.2	ℍℍ
wood wax	0.35	6.2	39.4	++	6.4	30.0	++
olive oil	1 cc	6.0	183.5	ℍℍ	6.4	230.8	ℍℍ
oleic acid	1 cc	5.8	111.6	ℍℍ	6.0	71.8	ℍℍ
stearic acid	0.5	6.2	48.6	ℍℍ	6.2	27.0	++
palmitic acid	0.5	5.8	49.0	ℍℍ	6.2	28.0	++
myristic acid	0.5	6.0	29.2	++	6.2	34.0	++
lauric acid	0.5	5.8	8.0	±	6.0	69.0	ℍℍ
capric acid	0.5	5.4	5.0	±	8.0	194.0	ℍℍ
cetyl alcohol	0.5	6.4	21.4	+	6.6	14.0	+
glycerol	1 cc	8.0	304.0	ℍℍ	8.2	188.6	ℍℍ
cholesterol	0.5	6.2	0	—	6.0	0	—
carbowax 400	0.5	5.8	1.0	±	6.0	0	—
carbowax 4000	0.5	6.2	0	—	—	—	—

weight of mycelium was measured with precautions described above. The amounts of wax or paraffin left unused in the medium were determined by extracting with chloroform. The efficiency of growth was computed as the ratio: mycelium harvested (mg dry weight)/carbon source consumed (mg) (Table 7).

The results with the wax-inhabitant W1 are almost self-evident and need no further explanation, the yield in mycelium steadily increasing with duration of culture and also in line with the consumption of either carbon sources added. (Fig. 1) The efficiency of growth as defined above amounted in average to about 0.51 on paraffin and 0.46 on beeswax.

The results with Aspergillus flavus are somewhat complicated by the circumstances that the growth of the mould as measured by the increase in dry weight of the harvested mycelium was fairly rapid only in the first week of culture, no appreciable gain being achieved in later periods. Also in this instance the consumption of added paraffin assumed a steady increase with incubation time, if we are to overlook fluctuations which were most probably due to the physical inhomogeneity of growth media with such substances as paraffin or wax. The decrease
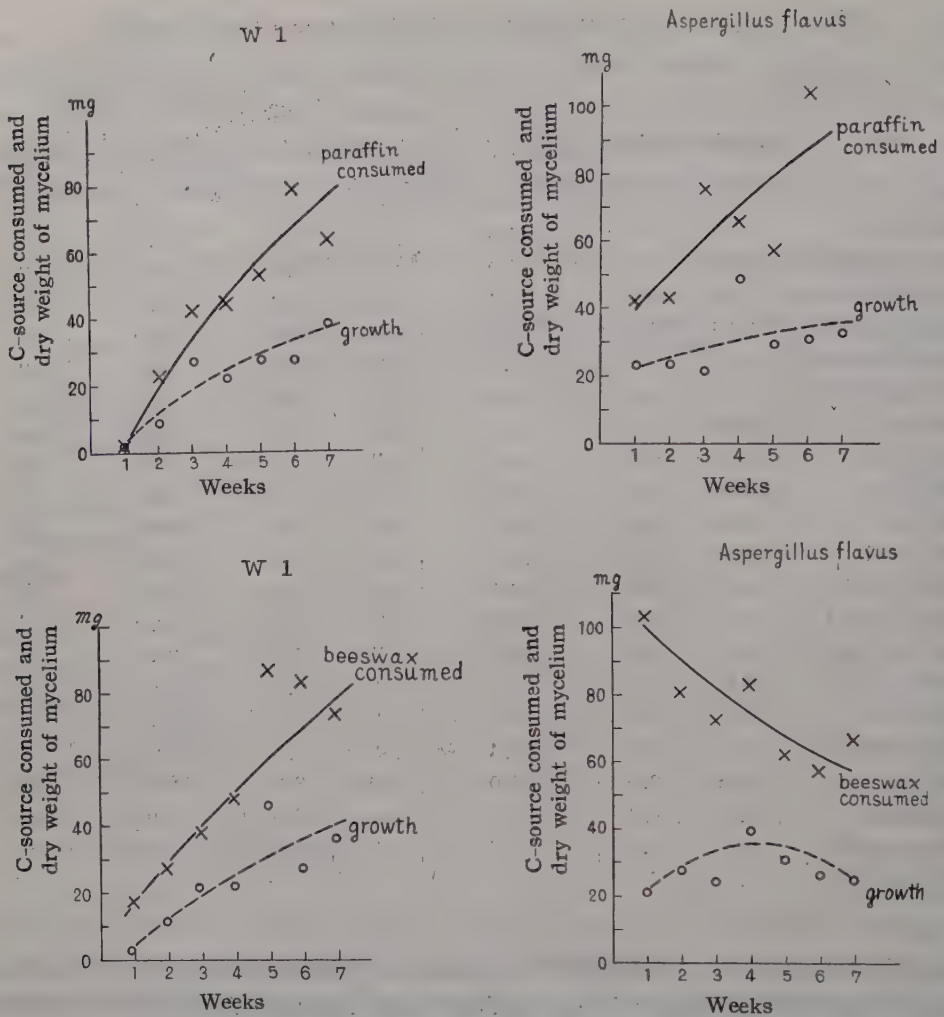
Fig. 1.　Development of mycelium on wax and paraffin.

in the computed values for the consumed amounts of wax with the duration of culture in this case is difficult to explain.

V)　*Effect of surface active substances.*

Several papers[16),17)] have appeared describing accelerating effect of surface active substances (e. g. Tween compounds) on the growth of microbes. In the following experiments Tween 40 and Tween 80 were tested in this respect. To 100 ml of the liquid culture medium containing wax or paraffin as usual in the present study, 0.05 ml of Tween 80 or Tween 40 was added (Table 8).

These surface active agents were found to be utilized by Strain W1 as the sole source of carbon and abundant growth, even outdoing that with paraffin, took place in their presence. Simultaneous presence of these substances together with paraffin

Table 7. Development of mycelium on wax and paraffin (liquid culture).

| C-Source | Incubation time (Weeks) | W 1 | | | | Aspergillus flavus | | | |
		End-pH	Dry wt. of mycelium (mg)	C-Source consumed (mg)	Efficiency of growth	End-pH	Dry wt. of mycelium (mg)	C-Source consumed (mg)	Efficiency of growth
Paraffin	1	6.0	1.4	0	——	6.0	24.0	0	——
	2	6.2	9.2	22.4	0.41	6.2	24.4	41.3	0.59
	3	6.2	27.2	42.1	0.65	6.0	22.4	43.2	0.52
	4	6.2	21.8	44.8	0.49	6.8	49.4	75.3	0.66
	5	6.2	28.0	53.2	0.53	6.8	30.0	65.9	0.46
	6	6.2	28.0	78.4	0.37	6.8	31.0	57.3	0.54
	7	6.4	38.0	62.4	0.61	6.8	32.4	104.5	0.31
Beeswax	1	6.0	3.0	17.4	0.17	6.4	21.4	104.3	0.21
	2	6.0	11.4	27.0	0.42	6.2	28.0	81.4	0.34
	3	6.2	22.0	38.5	0.57	6.4	25.0	73.3	0.34
	4	6.2	21.8	48.1	0.43	6.8	40.0	84.3	0.48
	5	6.4	46.2	87.5	0.53	6.8	31.0	62.0	0.50
	6	6.8	26.8	84.2	0.31	6.8	27.0	57.9	0.46
	7	6.8	36.2	72.9	0.50	6.8	25.0	67.2	0.37

resulted in mycelium yields remarkably superceding either individual levels, but appreciably lower than the sum of figures to be obtained with the individual ingredients. *Aspergillus flavus*, on the other hand, was found to be incapable of growing on either of these surface active agents; the addition of Tween 40 to the paraffin culture medium had no effect whatsoever on the yield. A set of shake-cultures also was carried out on the same media, with essentially the same results as above.

Table 8. Effect of surface active substances (liquid culture).

| C-source | Dry weight of mycelium (mg) | |
	W1	Aspergillus flavus
none	3.4	0
sucrose	934.0	354.8
paraffin	82.0	51.8
Tween 80	145.2	——
Tween 80+paraffin	172.0	——
Tween 40	204.0	4.0
Tween 40+paraffin	240.0	54.6

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Summary

1) Twenty-two strains of moulds were isolated from the culm of bamboo plants and tested for their ability of growing on wax and paraffin which were added as the sole source of carbon.

2) Laboratory stock of ordinary mould species also were examined in this regard.

3) It was revealed that "cerophilic growth" i.e. the growth on wax, paraffin etc. which were added as the sole source of carbon was a feature not common among mould forms in general.

4) Allied substances other than wax and paraffin were also tested and found to be effective in supporting the growth of some of the moulds investigated.

5) Cerophilic growth on wax and paraffin was followed with cultures of Strain W1 and *Aspergillus flavus* in regard to the increase in weight of mycelium and the consumption of added carbon sources was simultaneously measured over a period of seven weeks of incubation.

6) Effects of surface active substances on the cerophilic growth of Strain W1 and *Aspergillus flavus* were investigated. The growth of the wax-inhabiting organism, W1, on paraffin was favorably affected by the addition of Tween 40 or Tween 80 to the culture medium.

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アカパンカビの子実体形成 I
培養濾液の被子器形成作用について

伊　藤　太　郎*

Taro ITO*: Fruit Body Formation in Red Bread Mould, *Neurospora crassa* I.
Effect of Culture Filtrate on Perithecial Formation

緒　　言

二極性菌類の有性生殖において, すなわち *Phycomycetes* では半数世代葉状体が相会し, 又は独立で造精器や生卵器をつくり, そして接合胞子並びに卵胞子形成へと進む過程, また *Ascomycetes* 並びに *Basidiomycetes* の二次菌糸形成とそれにつゞく子ノウ胞子並びに担胞子形成の有性生殖過程において, 配偶子器形成並びに菌糸融合胞子形成がどの様にしておきるかということについては細胞学的にしらべられたものを除いてあまり例をみない。 *Phycomycetes* では *Achlya bisexualis* 並びに *A. ambisexualis* の同種内及び異種間の雌雄菌糸相互に配偶子器形成を階段的に促進させる現象があって雌性菌糸の液体培養濾液は雄性菌糸に造精器形成を, また雄性菌糸濾液は雌性菌糸に生卵器形成を促がす。 まだ造精器形成をみない雄性菌糸の液体培養濾液には雌性菌糸濾液の作用をたすけるはたらきがある[4]。 *Ascomycetes* では *Bombardia lunata*, *Glomerella cingulata*, 及び *Neurospora sitophila* で, また *Basidiomycetes* では *Hypomyces solani* と *Lenzites betulina* とでその子実体形成に対し培養濾液がいかなる影響をもつかがしらべられている。 かゝる研究は雌雄接合型が子実体をつくるさい, その相互間に起る諸現象を解析してゆく上に妥当なものと考えられるが, 二, 三の例を除いて充分な知見は得られていない。 Dodge[1] は *Neurospora sitophila* で単一性型菌糸濾液が反対の性型菌糸に被子器をつくるかどうかを調べ, 好気的に両型が混合された場合に限り被子器形成がおこることを報告している。 Zickler[5] は *Bombardia lunata* で, 同様な実験を行ったがやはり否定的結果に終っている。 しかし最近, Driver と Wheeler[2] は *Glomerella cingulata* のホモタリックな一系統について, その培養濾液 (燕麦餅抽出液加用培養基を濾液源として) が被子器形成を促進することをみている。

筆者はアカパンカビ *Neurospora crassa* の培養濾液がその子実体形成に有効に作用し, その混合系濾液 (雌雄両系を同時に培養して得た濾液) は単一系濾液 (雌雄両系のうち一方だけを培養して得た濾液) にくらべ有効であることをみたので[3] その有効性について, 1) 混合系濾液の効果は単一系濾液の効果の加されたものか, を追求し併せて 2) 濾液の効果は, 子実体形成のどの過程に有効に作用するか, を解明するため実験を行い得られた二, 三の結果をここに報告する。

材　料　と　方　法

本実験に用いた材料は, 寒天基礎培養基に継代保存した *Neurospora crassa* 野生型の 4A (＋系, 以後 A 系とよぶ) 系統と 8a (－系, 以後 a 系とよぶ) 系統で菌糸並びに分生胞子に外部形態的異常のない, かつ適時交配して子実体形成のみられるものである。 実験に用いた培養濾液の調製は, まず 100 ml エルレンマイヤー・フラスコに殺菌前 pH6 に規正した液体基礎培養基 50 ml をいれ, これに分生胞子 (混合系濾液の場合には A 系並びに a 系を, 単一系濾液の場合には A 系又は a

* 帯広畜産大学酪農学科植物学教室 Botanical Laboratory, Dairy Section, Obihiro Zoo Technical College, Obihiro, Japan.

系を殺菌水に懸濁したもの）懸濁液一白金耳を接種し 26 ℃ 暗室恒温箱に 72 時間保った後濾過して生育菌体を除いた。培養濾液調製のために用いた基礎培養液は蒸溜水 1 l 中酒石酸アンモニウム 5 g，硝酸アンモニウム 1 g，リン酸第一カリウム 1 g，硫酸マグネシウム 0.5 g，塩化ナトリウム 0.1 g，塩化カルシウム 0.1 g，ショ糖 15 g，微量重金属を含むものである。

子実体形成検定は滅菌後まもない 10 ml 寒天培養基の表面に，上記方法で得た濾液 1 ml を，対照区では濾液の代りに無接種のまゝ 26 ℃ 暗室恒温箱に 72 時間保っておいた基礎培養液 1 ml

を添加し，固化したものに，A 系は a 系を接種して 26 ℃ 暗室恒温箱に 15 日乃至 20 日保った後形成された被子器数を未成熟のもの，成熟のものすべてについて計数した。

結　果

培養濾液を添加した場合の寒天の斜面上の被子器形成は第一図並びに第一表の如くで，A 系濾液添加は a 系濾液添加よりも有効，混合系濾液添加の場合には更に被子器数はまし，単一系濾液添加と混合系濾液添加の差は有意である（P=0.01）。即ち混合系濾液添加の場合が最も有効であった。

Fig. 1.　基礎培地に A 系濾液を添加したときの子実体形成

Fig. 2.　a 系濾液の場合
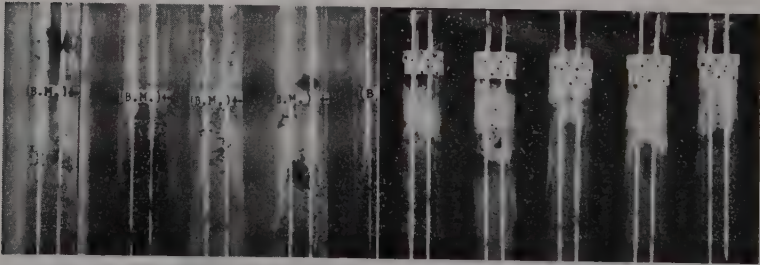
Fig. 3.　A, a 系混合濾液の場合

Fig. 4.　子実体形成用培養基に基礎培養基添加の場合

第 1 表　混合系単一系濾液添加による形成被子器数

培養濾液源	形　成　被　子　器　数							
	1	2	3	4	5	合　計	平　均	P　値
A, a 系（混合系濾液）	84	130	128	135	102	579	116	0.01
A 系　（単一系濾液）	93	95	85	79	108	460	92	0.3〜0.2
a 系（　〃　）	14	12	18	35	52	131	26	0.3〜0.2
対　　　　　照	25	58	2	8	30	123	25	0.3〜0.2
A 系　〜　a 系	79	83	67	44	56	329	66	0.01

次に A 系及び a 系単一系濾液の量比を 10:0,
9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8,
1:9, 0:10 とかえて 添加した 各種培養基につい
て被子器形成の良否を試験した。これはA系並び
に a 系単一系濾液による被子器形成促進と，A系
a 系混合系濾液による促進との間に，A系並びに
a 系単一系濾液がどんな作用をもつかを調べるた
めである。被子器数は混合系濾液の場合が406で
最大でA系濾液 0.3 ml a 系濾液 0.7 ml 附近に
おいて最低を示した。

次に二次菌絲形成に始まり子ノウ胞子形成に終
る子実体形成過程において雌雄接合型にいかなる
作用がみられるか，単一性型を混合系並に単一系
濾液添加の寒天斜面培養基に接種した。即ちA系
及び a 系を各別々に（i）混合系濾液（ii）A系濾
液（iii）a 系濾液を夫々加えた三種の斜面培養基
に接種して被子器形成並びに子ノウ胞子形成の有
無を調べた。なお対照としては基礎培養基 1ml 添
加の子実体形成用培養基を用いた。（i）では共に
被子器外皮のみ形成されたが子ノウや子ノウ胞子
はできない。（ii）では a 系を（iii）では A 系を接
種した場合のみ即ち反対性型の濾液添加の場合の
み（i）と同様な被子器が形成された。対照ではい
ずれの場合も被子器形成はみられなかった。これ
らの結果は被子器外皮形成はその反対性型の濾液
により誘起されることを示すものと考えられる。

考　察

二極性菌類において半数世代葉状体の培養濾液
の被子器形成作用としてはDodge が *Neurospora
silophila* を用いてその単一性型菌絲に，反対性型
菌絲の肉汁培養濾液が被子器をつくるかどうかた
しかめているが，否定的結果に終っている[1]。

第2表　混合系濾液 並びに 単一系濾液の
各種量比混合による 形成被子器数

培養濾液源		形成被子器数
A, a 系（混合系濾液）		406
A系 1 ml	a 系 0 ml	330
0.9	0.1	365
0.8	0.2	338
0.7	0.3	207
0.6	0.4	168
0.5	0.5	196
0.4	0.6	133
0.3	0.7	113
0.2	0.8	
0.1	0.9	229
0	1	287
合　計		2365
平　均		237

※ 左端に縦書き「A系並びにa系濾液混合量」

筆者の実験によれば，雌系濾液は雄系菌絲に雄系
濾液は雌系菌絲に被子器を形成し子ノウ並びに子
ノウ胞子を形成しなかった。雌雄接合型を接合さ
せるとき濾液源として基礎培養基を用いた場合に
は必らず被子器は形成されるが濾液源培養基の窒
素源として硝酸態のもの，亜硝酸態のもの，アン
モニア態のもの，更に有機態のもの，とかえた場

第3表　単一性型を単独に各種培養基に接種した場合の被子器形成の有無

培養濾液源 ＼ 接種単一性型	A　系	a　系
A, a 系（混合系濾液）	被子器形成	被子器形成
A 系　（単一系濾液）	被子器形成せず	被子器形成
a 系　（ 〃 ）	被子器形成	被子器形成せず
対　照	被子器形成せず	被子器形成せず

合の被子器形成の効果にはたしかに差がみられる
（未発表）ので，単一性型菌絲に対する，単一系濾
液の被子器形成効果も，空素源の種類により変化
すると考えられ，おそらく Dodge の場合は，培
養濾液源並びに子実体形成の培養基の組成が影響
しているのではあるまいかと考える。

雌雄接合型に対するつまり受精接合に対する影
響としては混合系濾液が単一系濾液にくらべ被子
器形成に有効に作用する（第1表）。これは第3表
に示した様に単一系濾液がその反対性型菌絲に被
子器をつくるということと，第2表に示した様に
単一系濾液を各種量混合した場合被子器数がいず
れも，混合系濾液添加の場合にくらべて劣ること
から混合系濾液の効果は単一系濾液の効果が相加
され，そしてそれに別の要因による形成促進効果
が加わった結果であると考えられる。別な要因に
ついてであるが，Raper が Achlya で行った実験
では雄型菌絲が生産する分泌物質は雌型菌絲の分
泌物質の造精器形成作用を助長するというが[4]二
つの単一系濾液が相互にその作用を助長すると
すれば，単一系濾液を混合した場合にも効果がみ
られる筈であるが，第2表にみる通り本実験結果
では妥当でない。別な第二の要因として考えられ
るものはヘテロカリオーシスである。Driver 及

び Wheeler 等[2] は *Glomerella cingulata* の自
家稔性系統の培養濾液が同系統の被子器形成に
有効であることをみている。自家受精的被子器形
成は核融合（karyogamy）が活潑におこる場合に
形成される活性物質によって著しく促進され又そ
の活性物質は核融合に達する前に処理してはじめ
て被子器形成に有効であることをみて，核融合に
よる活性物質の生成と被子器形成即ち核融合に対
する有効性を認めている。従つて混合系濾液の効
果も雌雄接合型の接合のときにおきると予想され
る異核混合（ヘテロカリオン）の形成とその子実
体形成への影響をしらべることによりこの要因は
明らかになるかも知れぬと考える。

最後に第1表並びに第2表に示した様に単一系
濾液の効果には差がみられる。即ち第2表にみる
様に単一系濾液の量を相互にかえた場合みられる
効果が A 系濾液 0.3 ml a 系濾液では 0.7 ml 附
近で最低を示したことから単一性系濾液の効果に
差があることが予想されるが，これは雌雄接合型
の本質的差異の問題と関聯して興味あることと思
われる。

終りにのぞみ本稿を草するにあたり懇切な御教
示と論文校閲を賜つた東京大学理学部植物学教室
田中信徳博士に深甚なる謝意を表す次第である。

Résumé

Effect of filtrate of culture medium containing mycelia of *Neurospora crassa* on the perithecial formation has been studied. The filtrate of medium containing mycelia of a single sexual strain of either mating types was less effective compared with that of medium containing mycelia of both mating types in stimulating the perithecial formation. The effect of the mixed culture filtrate surpasses the additive effect of two single culture filtrates of each mating type. This seems to indicate the presence of cooperative unknown other factor. In fact, the single culture filtrate of one mating type stimulates the formation of perithecia on mycelia of the opposite mating type; these perithecia, however, have failed to produce asci and ascospores. Consequently, it may be concluded that the formation of perithecia on the haploid vegetative thallus of one mating type are stimulated by diffusible substance secreted by mycelia of the opposite mating type, quite irrespective of the sexual fusion of nuclei of both mating types.

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第21回大会講演

特 別 講 演

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会 長 講 演

服 部 静 夫：シソ科及びゴマノハグサ科の少
糖類について

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る研究

曾根田正巳：本邦産 Cryptococcus 属菌について

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広瀬 弘幸：ヨツメモの性と有性生殖

千原 光雄：邦産ベニマダラ属の生活環

梅崎 勇：日本産藍藻 Symploca hydnoides に
ついて

黒木 宗尚：アマノリ類の生活史の研究 続報
オオノノリの繁殖器官について

瀬木 紀男：アオノリの海に於ける発芽について

田中 剛：原始紅藻類ニセウシケノリについて

鈴木 兵二：日本産オオミズゴケ類について

越智 春美：北海道におけるカサゴケ科の蘚類
（予報）

川崎 次男：ノキシノブ属 (Lepisorus) 数種の有
性世代

松川 昌弘：スギナ類についての一考察

清水建美・北村四郎：日本の石灰岩植物

栗本 喬：花粉の発芽率からみた松柏植物

小山 鉄夫：シンジュガヤ類の小穂構造について

小宮 定志：タヌキモ科植物の腺発生について

菅原 繁蔵：樺太植物の総括

細川隆英・小村 精：Interspecific correlation
による着生植物の群落構造解析（続）

細川隆英・小谷信矢：着生植物の樹上分布と日補
償点について

細川 隆英：Ulota crispula の生態

中西 哲：岩上着生蘚苔群落の生態

斎藤 実・佐々木太一：北海道の蘚苔類 (2). ミ
ズナラの着生蘚苔類

野田 光蔵：遼東半島沿岸の海藻相について

奥富 清：宮島の針葉樹林とその立地条件

飯泉 茂：ドクダミの群体形についての考察

宮脇 昭：北海道の雑草群落

小野 幹雄：裏日本植物の分布型

村田 茂三：麦畠の微細気象の観測と研究 第
2 報 分蘖最盛期における観測結果

奥野 春雄：岡山県八東村の珪藻土について

三木 茂：遺体からみたチョウセンゴョウにつ
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生嶋 功・篠崎吉郎：新しい方法による生長曲線
の解析

小川房人・依田恭二：個体密度の自然間引

岡田幸郎・桃谷好英：マツ科における種子蛋白の
変異

相馬 寛吉：仙台附近鮮新世亜炭の花粉分析（第
二報）

高橋 信夫：山形県内に点在する二, 三炭坑の花
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高橋基生・松浦正郎：植物の健康並に性能の診断
法としての根系呼吸測定法

高橋基生・谷本義男・青木和子：植物の生長経過
別による培養液組成の根系呼吸に及ぼす影響

高橋 基生：切断根系の養分吸収並に呼吸の不自
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高橋基生・谷本義男・平田コノ：各種水耕法の性
能判定と根系呼吸

高橋基生・石黒 勇・桝井 孝：根系呼吸に対す
る土壌成分の温度補償性と植物の分布

高橋基生・青木喜昭：青枯水田土壌の特色

堀川 芳雄：ブナの植物地理学的研究 (I) 日本
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佐々木好之・堀川芳雄：ブナの植物地理学的研究
(II) 中国地方におけるブナ林と照葉樹林の推
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村田 芳蔵：夕張市（夕張岳を除く）の植物につ

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堀田　康雄：子葉の離層分化と遊離 SH 基及び生長素

古谷　雅樹：オーキシン処理によつて見出されたインゲン子葉の形態形成における役割

古谷雅樹・相馬研吾：インゲン幼胚の組織培養におけるオーキシン処理効果

山田　晃弘：トウゴマ発芽初期における胚組織内のリパーゼ

馬場　三吾：癒傷組織形成期間中の組織の呼吸について

岩波　洋造：雌ずいの澱粉と糖について

飯島　衛：還元分裂中における微量 Zn の組織化学的研究

八戸　正夫：ユキノシタの異なる表皮細胞間の原形質分離限界濃度の比較

植田利喜造・村上　悟：植物細胞における色素吸收の定量的測定

島村　環：裸子植物の受精及び前胚の顕微化学的研究

伊倉伊三美：染色体による羊歯類精子及び前葉体の荷電と rH について

平岡　俊佑：フォイルゲン核アルデヒド反応に関する二，三の知見

植田利喜造・前田　微：螢光顕微鏡による植物細胞の研究

山崎　典子：コアツモリ (Cypripedium debile) の減数第一，第二分裂中期染色体の退色反応について

水野　忠款：カキラン (Epipactis longifolia) の花粉粒分裂の同時性について

木村　劼二：帽菌類の diploidisation における移行核について

佐々木正人：車軸藻類における細胞学的研究 IV. ケナガシャジクモ (Chara Benthamii) と，カタシャジクモ (Chara globularis) における造精糸の細胞数と分裂の頻度との関係

大山　正：X-線の高度照射における細胞分裂の特異性. (IV) 飽和影響後における分裂

寺田　保：電子顕微鏡による変形菌の微細構造に関する研究 (II)

植田　勝巳：Euglena gracilis の電子顕微鏡による観察

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田中　隆荘：ナカガワノギクの生態型に関する形態学的細胞学的研究

下斗米直昌・益森静生：ノジギクの種内倍数性と地理的分布

藤原悠紀雄：Aster 属植物の核型分析 (第 4 報)

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柴田　寛三：Reed Canary Grass における異数性と核型

稲田朝次・岩佐正一：異質細胞質による Chlorophyll 形成欠陥

田村半吾・菅原繁蔵・浅利正俊：果樹接木による栄養交雑

小野記彦・酒井文三：ヤクシソウ×アキノノゲシの F1 における染色体減少の原因 III

寺川　博典：ヒラタケの発生

斎藤真太郎・下瀬　敏：細胞の蒴歯の発生学的研究

伊藤　道夫：モエジマシダ前葉体細胞の再生能及び分裂能について

竹内　正幸：インゲンの胚発生 (2)

木村陽二郎：アマの子房構造

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原　襄：ツツジ科の生長点

亘理俊次・小倉　謙：Xenoxylon latiporosum に関する二，三の知見

和田　文吾：紡錘体に関する二，三の問題

渡部一郎・高沖　武：一時的及び継続的通電の発根促進効果

柴岡　孝雄：車軸藻類の細胞間興奮伝導

小田　健二：フラスモの膜電位とその時定数

今村駿一郎：アサガオ子葉の日長感応

滝本　敦：ムシトリナデシコの開花を促進する光週期の明期における二つの段階について

池庄司幸江：花粉の発芽に及ぼす X-線の影響について

高須　謙一：植物生理学上より見たる日長について

尾形　英二：コンコセリス糸状体の紫孔成長について

西田晃二郎・相馬悌介・日比野信一：気孔の夜間開孔と葉の有機酸との関係について

玉井直人・西田晃二郎：葉の P32 蓄積に及ぼす光

るフォスフォリラーゼ, 燐酸, pH, 等電点, 澱
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相見霊三・村上　高：水稲の種実における澱粉の
集積機構に関する細胞生理的考察

石川　重夫：種子の発芽に伴う呼吸におよぼすク
マリンの影響

柴田　治：光過処理に伴うある代謝系の活性度
変化

平岡　純一：酵母変異菌のイオン交換性 [1]

高田　英夫：酵母変異菌のイオン交換性 [2]

持塚　洵・芦田譲治：銅抵抗性細胞の起源につい
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内貴信夫・芦田譲治：銅抵抗性コウボ菌の硫黄代
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芦田譲治・今井六雄・榊原浄子・内貴信夫：銅抵
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柳島直彦・永井　進：酵母の W 変異と銅適応

村山徹郎・芦田譲治：コウボのアミノ酸プールに
対する諸毒物の影響

荒勝　豊：酵母の銅耐性と窒素源

近衛　麻也：メタセコイアの生育と環境条件（温
度と光）について

加藤　次郎：ジベレリンと生長調節物質との相互
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賀来　章輔：長日植物ベッチ（Vicia sativa）の光
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小西　通夫：Auxin 処理によるムシトリナデシ
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小林静子・芦田譲治：オーキシン作用とペクチン
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須田省三・新　勝光：マレイン酸ヒドラジドの作
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長尾昌之・大脇頼子・草野晴子・尾辻　望：トラ
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増田芳雄・高田英夫：オーキシン作用の原形質学
的研究 I

小清水卓二・吉原朝子：イヌマキの胎生種子及び
休眠種子内における生長作用物質の動静

小林　万寿男：茎の極性と生長素

岩田修造・須田省三：二, 三緑藻のつくる生長素
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中沢　敬止：エンドウの伸長生長における IAA
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村上　進：フジアザミのポリフルクトサンにつ
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木下　広野：シラネアオイの根茎の成分

本　会　記　事

第 21 回　大　会

　昭和 31 年 7 月 9 日（月）〜18（水）の 10 日
間にわたつて札幌市を中心に開催された。9 日〜
11 日は見学 E 班, 11 日の晩は評議員会, 12,
13 日は一般講演並びに各部会, 14 日は 特別 講
演・総会・会長講演・公開講演・懇親会と予定通
り事が運ばれ, 14 日の見学 A 班, 及び 14 日
15 日に出発した見学 B, C, D 班も夫々予定通
り無事解散し, 大会の行事はすべて盛会裡に終了
した。7 月中旬の北海道は日本中でももつとも美
しく快いところといわれているが, 今年の異常低
温は参加者を驚かせた。参集した会員は道外道内
をふくめて 400 名を越え, これに 準会員や会員

外の聴講者を加えると, 予期以上の参加者数で主
催者側をよろこばせた。一般講演は申込数 187
で, 会場は A（農学部 農芸化学科一階講堂）B
（農学部農芸化学科二階講堂）, C（理学部南講義
室）及び D（理学部北講義室）の 4 カ所が用い
られた。いずれも北海道大学関係のものである。
一般講演は申込みのうちいくつかは中止となり又
いくつかが追加され実際に行われた数は別欄のよ
うに 176 であつた。一般講演の区切り毎に行わ
れた総合討論は極めて活発であつて, 次に行われ
た各部会における討論と共に学会の行き方に対し
何等かの示唆を与えるものがあつた。記念撮影は
12 日正午に農学部正面玄関前で行われた。14 日
には農学部大講堂において特別講演に次いで総会

が行われ，続いて会長講演がなされ，これをもつて大会の主行事である学術講演は終了した。その後見学 A 班の出発と同時に，同じ農学部大講堂で公開講演会が開催された。懇親会は産業会館で札幌市長招待会を兼ねて盛大に行われ，その後見学 C，D 班が札幌駅発，15 日朝は見学 B 班が出発した。

この大会にあたり，北海道庁，札幌市，室蘭市，函館市，網走市等の心からなる援助をうけた。また道内の有力なる商社多数の厚志による多額の寄附金をうけた。大会の運営がスムーズに運び，参会者に多大の満足を与えることができたのも，大会の役員として活躍された北海道支部の方々のお蔭であるのは言を俟たないが，道内の官民多数の理解と援助の賜であることを銘記するものである。

評議員会（7 月 11 日午後 6 時半，北海道大学理学部会議室）

出席者　評議員 12 名（欠席 10 名），会長，幹事長，幹事 5 名。

服部会長の挨拶につづいて次の諸事項の報告および承認が行われた。役員移動，現在会員の状況，会員移動，図書の寄贈・交換等，前回の評議員会のとき議題となつた学会図書の一部地方支部移管，会計決算・予算，編集の状況。なお，編集委員への謝礼は 1 編 200 円に変更された。

ついで，次の諸事項の協議および決定が行われた。

（1）会則一部変更の件。付則第 3 第 3 条が現状にそぐわないため，「幹事長・幹事・編集委員はいずれも会長が指名する。」と変更する原案を総会に提出する。

（2）創立 75 周年記念事業の件。創立 75 周年記念大会を来年秋東京で行う。大会会長に小倉謙氏を推し，国外，国内の学会からメッセージをもらい，記念講演を行う。また，名誉，特別，外国通信会員の推薦を行う。

なお，このほか，記念事業として植雑索引（第 46 巻以降），記念号，記念写真集等の出版が協議されたが，いずれも経費の点から無理であることが認められた。ただ，学会の図書目録は来年度内に植雑に掲載することになつた。

（3）学会会員名簿発行の件。昭和 31 年 12 月

31 日現在で会員名簿を作成し，来年 1 月号と同時に発送する。この名簿には 31 年度会費を 1 期以上おさめた人を載せる。

（4）会長・評議員選挙の件。上記の名簿を有権者名簿として来年 2 月頃に投票を行う。

（5）各種候補者推薦の件。

A.　次期会長候補者：服部静夫，篠遠喜人，松浦一，郡場寛，木原均。

B.　日本学術会議第 4 期会員候補者：服部静夫，松浦一。

C.　文部省学術奨励審議会科学研究費等分科審議会委員候補者：大槻虎男，本田正次，和田文吾の順。

D.　毎日学術奨励金候補研究：渡辺篤，柴岡孝雄。

以上の推薦を決定した。このうち，B は選挙規則によつて推薦された者の承諾がなければ候補者に推すことができないので，両氏が承諾されるかどうかを後日幹事長に申し出ることになつた。またC は文部省から推薦を依頼してくる人数が年によつて 2 名のときと 3 名のときとがあるため，一応順位をつけて 3 名候補者を挙げておくことにした。

総　会（7 月 14 日午前 10 時，北海道大学農学部大講堂）

服部会長の挨拶の後，会長司会のもとに次の諸事項の報告，承認および議決が行われた。

（1）役員移動の報告および承認（幹事長，評議員：3 月号掲載，幹事，編集委員：4 月号掲載）。

（2）現在会員の状況報告（昭和 31 年 6 月 30 日現在：1124 名。うち名誉会員 7，特別会員 26，外国通信会員 4，終身会員 50，通常会員 1037）。

（3）会員移動の報告（昭和 30 年 10 月 1 日—昭和 31 年 6 月 30 日：死亡 5，退会 15，除名 37，入会 120。差引増加 63）。

（4）図書の交換，寄贈，その他の状況報告（交換：国外受理 82，発送 78，国内受理 39，発送 39，寄贈：国外受理 4，発送 2，国内受理 16，発送 1，其他受理 1，発送 3，予約購読 209）。

（5）昭和 30 年度決算の報告（昭和 30 年 4 月—12 月：2 月号掲載）。

（6）昭和 31 年度前半決算の報告および後半予算の説明（別表の通り）。

（7）評議員会の報告（前記の通り）。

（8）会則変更。会長より付則第3第3条変更に関する評議員会原案が提出され、議論されたが、印東弘玄氏が提案された次のような修正案が賛成 64、反対2 で可決された。

付則第3第3条　幹事長・幹事・編集委員はいずれも会長が依嘱する。

つづいて会長の要望によつて来年度東京で開催される創立75周年記念大会の行事について、一般会員から希望が述べられた。この際、一般講演と並行してシンポジウムを行うことの希望が多く、シンポジウムに関しての種々の問題が論議され、また要望が述べられた。

服部会長はシンポジウムの形を円滑にするようよい計画をたてる努力を約し、総会を終つた。

31 年度前半（1～6 月）決算および
後半（7～12 月）予定

［収　入］	（前半決算）	（後半予定）	（全年度予定）
会　　費	523,983	380,000	903,983
予約購読料	174,977	65,000	239,977
一　部　売	700	—	700
バックナンバー	16,650	—	16,650
印　刷　代	75,281	20,000	95,281
利　　子	3,445	2,000	5,445
広　告　料	12,250	10,000	22,250
小倉記念会より	100,000	—	100,000
小　　計	907,286	477,000	1,384,286
前年度より繰越	257,010	—	257,010
総　　計	1,164,296		1,641,296
［支　出］			
出　版　費	610,813	495,000	1,105,813
発　送　費	82,324	42,000	124,324
編集関係費	48,141	40,000	88,141
図書関係費	16,270	30,000	46,270
庶務関係費	40,985	39,000	79,985
大会関係費	30,695	20,000	50,695
支部補助	10,000	10,000	20,000
幹事手当	36,000	30,000	66,000
小　　計	875,228	706,000	1,581,228
残　　高	289,068		60,068
総　　計	1,164,296		1,641,296

懇　親　会

7 月 14 日、市内の見学を終つた後、午後5時半から札幌市産業会館の大広間で開催された。参加者は来賓を含めて 200 名余り、会は札幌市長の招待会を兼ねて行われ、また日本ビール会社からは札幌ビールの寄贈を受けて卓上花を添えた。会は山田大会会長・札幌市長・杉野目北大学長・服部学会会長の順に挨拶があり、ビールを飲むほどに全国から参加した会員の遠くは鹿児島の方を含めてのテーブルスピーチがあり、終りには大会名誉会長伊藤誠哉氏の挨拶があつて後、同氏の発声で植物学会ののため乾盃をして会を一まず終了した。その後北海道庁・道教委・網走支庁からそれぞれ提供をうけた道内観光映画を観賞し、これから道内を旅行する人も、すぐに退道してしまう人々も共に北海道の自然の美しさを観賞した。終つて後、見学の C・D 班の参加者は会場から直接札幌駅へと向つた。

公　開　講　演

7 月 14 日午後 2 時から北大農学部大講堂に於て行われ、奈良女子大教授小清水卓二氏の『万葉植物に就て』と題する講演が行われ、又それに引続き日本新薬株式会社提供のミブヨモギに関する映画の上映があり、参会者 250 余名、4 時過ぎ終了、頗る盛会であつた。

関係諸学会

細胞形態部会

7 月 12 日、17 時より約 70 名の参加を得て形態細胞部会を開いた。17 時より 18 時迄、夕食をともにしながら、部会の運営等につき議し、18 時より、「形と系統の関係」、「形態形成と生化学」、「細胞の生体と固定における構造変化」及び「種の分化の研究」という四つの問題について、それぞれ前川文夫、古谷雅樹、和田文吾及び下斗米直昌の諸氏より話題の提供があり、討論が行われ、20 時 30 分閉会した。（司会：木村陽二郎、座長：熊沢正夫、島村環）。会場の準備、議題の選択等に不備の点はあつたが、参会者が活発な討論を展開したことによつて、各研究分野の問題点を明確にし、今後の部会の運営についても一応の方針が打出されたのはよい収穫であつた。

第 9 回植物分類学会

7 月 13 日、17 時 30 分～21時、農学部会議室、出席会員50名、会食、印東弘玄幹事の司会によつて会は進行された。先ず秋山茂雄氏から会員

に対し，歓迎の辞があり，議事に入つて印東弘玄氏の 30 年度庶務報告，加崎英男氏の会計報告がなされた。次に幹事側提案の議題 4 件は次の如く決められた。(1) Taxon の購入方法は幹事に一任すること。(2) 会員名簿を作製すること。(3) 南極観測に関して分類学会としての動きに意見のある会員は幹事まで知らせること。(4) 次年度の新幹事として次回開催地は東京であり，木村陽二郎(庶務)，福島博（会計）の両氏が推薦された。又分類学会編の「植物科名に関する標準和名表」は極めて正確である旨を津山尚氏からの伝言として木村陽二郎氏から会員に伝えられた。議事終つて，木村陽二郎氏の 「パリー土産話」（カラースライド），映画「宮部先生の日常のスケッチ」，幻燈，ガイド説明付「北大植物園」の紹介等が賑やかに繰広げられ和やかな雰囲気の中に散会した。

付記。当学会の会場は定山渓を予定していたが（分類学会々報第 5 号 4 頁掲載）都合により北大に変更した。又地元幹事館脇操氏が病気入院加療中のため出席できなかつたことは遺憾であつた，一日も早く平癒されることを望みます。秋山茂雄氏他地元会員が会合開催のため協力した。

植物生理学部会

7 月 13 日，18.30～21.00 商工会議所講堂にて，下記の 2 つのテーマについて討論された。

1) 植物の呼吸（座長：小倉安之・巌佐耕三），一般講演中から，特に KCN によつて促進される呼吸のメカニズム，末端酸化酵素，動物・植物・微生物間での呼吸酵素系の異同などについて，話題の提供及び討論があつた。 2) 生理学実習の現状とその在り方（座長：芦田譲治・真船和夫），各大学から簡単な報告があつた後，活発な意見の交換がなされた。結論を得るまでに到らなかつたが，今後真剣に検討すべき問題であることが痛感された。

第 4 回日本藻類学会

7 月 12 日，15 時～21時，札幌市北 3 条西 7 丁目・水産会館，出席会員 41 名，他に水産業者代表若干名。中村義輝幹事開会の辞を述べ，山田幸男会長より本会発足以来 3 年を閲しその基礎も漸く充実し現在 265 名の会員を擁し第 4 年目に入つたのであるが，一方国際藻類学会設立の機運もあり，米国加州大学パーペンフス教授の照会に対しては入会希望者は我会から 33 名あり今後益々本会の発展のために努力したい旨の挨拶があり，ついで議長選出に入り，例年に倣い開催地在住の会員中より選出することとし時田郁氏を選出後，川嶋昭二幹事の昭和 30 年度庶務報告，舟橋説往幹事の同会計報告がなされた。更に機関雑誌「藻類」に関する編集等を中心に活発な質疑応答が行われ特に会長，須藤俊造幹事より会誌の原稿が論文に偏重の傾向あり，総合抄録，雑録，雑報等の投稿を希望する旨会員に要望され，総会を閉じ講演会に移つた。講師，演題は次の通り。

〔1〕 沢村政成氏（道水産製品課）一戦前並戦後に於ける本道コンブ他海藻類の生産消流状況

〔2〕 中村義輝氏（北大海藻研）一コンブ増殖上の諸問題（幻燈使用）

講演終了後 10 分間休憩，北海道漁業組合連合会及び水産研究会共催の懇親及び座談会に移り，司会者時田郁氏，夕食を共にしながら自己紹介及び出席会員と水産業者とのコンブ，ノリ，アオノリ等の有用海藻類について各々の立場から意見の交換，質疑応答が行われた。なおこの席上における内容記事は近く発行の北水試月報に掲載される予定である。又日本藻類学会は毎年植物学会大会地及び水産学会大会地において上述の如き会合を開催することになつており，当学会の事務所は北大理学部植物学教室内である。

菌類談話会

7 月 12 日，18 時～21 時 30 分，北大農学部附属植物園にて。集まる者 59 名。印東弘玄氏のMucorales, Piptocephalidaceae に属する菌についての興味深い講演並びに伊藤誠哉氏の「マツタケ目の分類を終つて」と題する広範な知見を総括した同目の新しい分類方式に関する講演あり。質疑応答の後会食に入る。この間菌学今後の発展等に関し談論風発。終つて日本の菌学生みの親である故宮部金吾先生の生前の 1 日を写した 36 mm 映画並びに同先生の録音（共に北大農学部植物学教室所蔵）の公開あり，極めて盛会裡に散会した。

生 態 部 会

7 月 12 日午後 6 時 30 分～9 時 北大農学部会議室，参加人員 植物学会員 39 名，北海道生態学会員 3 名，計 42 名。

午後 5 時 30 分より会場に参集した会員は各自の意見を交換し談笑，6 時 30 分開会，当夜の運

営は北海道生態学会委員がこれに当った。林業試
験場中野氏が開会を宣し、次いで札幌市立病院に
入院中であった館脇博士が挨拶を述べ、出席会員
一同の賛成を得て吉井会長を議長に推挙した。
吉井博士の議事進行によって会はつつがなく和気
あいあいたる中に次から次へと進められ、話題提
供者細川隆英、佐々木好之、小川房人、佐伯敏郎、
飯泉茂、斎藤実の諸氏等が交々立って研究方針や
生態学に関する意見を述べ、列席諸氏各自の所見
を自由に論じ、諸題について活溌に論議した。年
代にこだわらず各自が真剣に意見を交換できた事
は非常にうれしかった。尽きざる名残りの中に楡
の木蔭の闇も全く深くなった 9 時頃散会した。

見 学

A 班（札幌市内及び近郊班）　138 名。

7 月 14 日　14.30　会長講演終了後、3 台のバ
スに分乗して農学部前を出発。先ず日本ビール会
社札幌工場を見学、冷たいサッポロ生ビールの饗
応にあずかり一同大満悦。時間のせくままに後髪
を引かれる思いを残して雪印乳業会社に向う。工
場を見学の後、ここでも天下の絶品雪印アイスク
リームの接待をうける。次いでバスは千歳街道を
経て月寒の種羊場（正しい名称は北海道農業試験
場畜産部）に到着。涯しない広野に声なく牧舎に
帰る羊群に北海道気分を満喫。しばしの休憩の後
北大農学部附属植物園に到り 17.10 解散。

B 班（支笏湖・登別・洞爺湖班）　19 名。

最初の申込みが 30 名をこえていたのに、いざ
となったらグンと減少して僅か 19 名となり、経
済的に主催者をあわてさせたが、数が少いだけに
一同極めて親密に楽しい旅をすることができた。

7 月 15 日　9.00　札幌駅前発の貸切バスで一路
支笏湖へ。湖は珍らしく晴れ、樽前山、恵庭岳をな
がめながら昼食。13.00 湖畔出発。途中白老のア
イヌ部落を見物、熊彫りのアイヌにカメラを向け
たとたんにモデル料を請求された人物あり。14.
30 登別温泉に到着。東洋一を誇る大浴場で汗を
流して、地獄谷を見物。その次は修学旅行団にわ
ずらわされない静かなグランドホテルでのんびり
と一泊。翌日は予定を変更して乗合バス、汽車を
利用して 11.00 洞爺湖温泉に到着。昼食後遊覧
船で中の島に到り森林博物館を見学、次いで世紀
の奇蹟と呼ばれる昭和新山を山麓から眺め、再び
温泉に戻る。お別れの晩餐の後、名残りを惜しみ
つつ 18.00 解散。遊覧船の事故のため湖上漂流
約 1 時間。救援艇出動の一幕もあり、一同和気藹
々、来年の学会には全員が集って湖上遭難(?)思
い出の会を開くことを満場一致決議をした。記念
事業として大島行きの案も出たが本日の遭難にこ

りてか、賛成者少く、結局浅草あたりを漂流しよ
うということになって、B 班はめでたく終末をつ
げた。

C 班（阿寒・川湯・網走班）　62 名。

7 月 14 日 21.00 札幌発夜行にて出発。翌 15
日朝釧路着、2 台のバスに分乗して出発。釧路市
中等見学後 13.00 阿寒湖畔着。沿道道路悪く夜
行の労疲と重なり一同グロッキーになる。天候曇
り丹頂鶴も見えず。午後 2 時より遊覧船で阿寒湖
上周遊。雲低く花少くまりもの姿も濁水の為覚束
なし。旅館は 1 人 2 畳平均で大分窮屈だったが一
行耐乏精神を発揮する。翌 16 日朝好天気の中に
横断道路、ペンケ湖、パンケ湖を通り午後 1 時弟
子屈着。途中バスを止め採集に努む。釧路川の青
さに興じ摩周湖に至る。霧をすかし妖麗な姿を満
喫後、硫黄山を通り川湯着。温泉の酸味に驚く。

17 日天候好し。和琴半島、屈斜路湖を廻り正午
美幌峠着。霧深く寒さ烈しく一行ふるえ上る。峠
を下れば忽ち快晴。17.00 網走着、博物館見学後
原生花園に至れば一面の花盛りは一行を喜ばす。
採集後天都山に登り雄大な夕陽を見る。市長観迎
宴に列し網走料理を賞味した後解散。

D 班（知床班）　35 名。

7 月 14 日：21.26 札幌発の列車はよく混んで
いたが、どうにか坐ることができたのは幸だった。

7 月 15 日：遠軽着の 4.15 は早すぎるし、中
湧別までのレールバスは小さくて窮屈。丁寧の朝
食はすばらしく、船で三里番屋へ。ドップシ着は
14.00 で、直ちに北見営林局の好意によるバスに
一同乗込む。道路修理のため大きく迂回し常呂に
出る。常呂の海浜植物群落をたずねた頃はオホー
ツクの海のガスが厚く包んでいた。能取湖を左手に
バスが走って網走労働会館に着く頃（20.00）は疲
れのためすやすや眠っていた方が多かった。

7 月 16 日：郷土博物館は各自で尋ねることに
して、原生花園に向ったのが丁度 10.00。網走バ
ス切つての案内嬢とバスで走るトウフツ湖岸の眺
めには一同感激。ハマナス、エゾキスゲなどが花
盛りとはいえぇオホツクの海風は冷たかった。午後
は網走湖―天都山―オホツク水族館を廻つて会館
へ（19.00）。

7 月 17 日：眠い眼をこすりながら 5.30 に会
館を出て巡視船 "天龍" が錨を上げたのが 7.00。
時速 14 ノットで、4 時間丁度で知床岬に着く。
この頃から低くたれこめていた雲がうすれ、国後
島の山々も見られ、上陸には手間どつたが、13.30
まで岬の草原に思い思いに採集を続ける。"天龍"
とはイオウで別れ、しばし断崖の近くをウトロま
で、最も恵まれた条件のもとで、小舟 2 隻に便乗
して走る。ウトロの岩からオホツクの海に沈む赤
い夕陽に、一同今度の見学旅行の幸多かつたこと
を感謝した。

7 月 18 日：午前中採集し船でオシンコシンま

で，再び北見営林局のバスで斜里着が 16.00。ここで再会を約して，名残を惜しみつつ解散した。

　E班（函館ニセコ班）22名。

　7月9日：　函館棧橋でヒヤヒヤして待つ内に 22 名集る。早速湯の川の営林局寮に旅装をとく。湯に入り朝食後女子トラピスト修道院をたずね，立待岬に向う。市長招待の昼食会に招かれて後，飛入り？ ラムネ工場視察後，函館山頂上に向う。港に出入りする連絡船の姿が白い航跡と共に眼にしみる。山を降りて七館の苗畑見学後，大沼公園紫水荘に 18.00 着。時折姿をのぞかす駒ケ岳の頂にカメラのシャッターが切られる（全員カメラ持参だつたので）。夜はボート，花火大会？。

　7月10日：　霧に深く包まれた大沼を後に，ディーゼルで森，森から列車で昆布へ，長万部で丁度午食。特製カニメシに舌つづみをうちつつ昆布着。ニセコ観光バスでチセハウスに向う。北海道

の代表的悪路につぐ悪路でハウスについた頃は一同クタクタ。夕食まで一同近くを散策し，夕食はそのままコンパになり，終つて花火大会。山小屋の一夜は嬉しいものになつた。

　7月11日：　朝早く山を狩太に降る。羊蹄山は雲に包まれていたが銀山トンネルを越すと陽が顔を出しはじめる。蘭島で下車し，忍路に向う。臨海実験所の船でカブト岩に渡り，ここで好意のサクランボのうまさに驚きながら昼食。札幌営林局のバスは国道を突走り小樽の苗畑に向う。見本林見学後，イチゴ畑に腰を下してよくくれたイチゴをむさぼり食う。日和山燈台をおとずれ，しばしの憩い。日本海上に浮ぶヨットの白い帆が美しかつた。手宮の古代文字見学後，バスは 60 km の時速でコンクリートの国道を札幌へ向う。18.00 に札幌着。短い日程ではあつたけど，長い時間を持つた気分で一同別れを惜んで解散。

会　員　移　動　（昭和 31 年 1 月〜6 月）

新　入　会

北　海　道

浅利　政俊　北海道松前郡松前町町立清部小学校・同松前町字清部 444

佐保　貴　北大理植

西山　保直　北大理植

三角　亨　北大農植

東　北

岩田　悦行　盛岡市上田岩手大学芸生物

相馬　寛吉　東北大理生物

関　東

会沢　正義　横浜市立大生物・横浜市西区平沼町 3 の 125

浅野　一男　長野県下伊那郡売木中学

越智　三五　世田ケ谷区深沢町 3 の 33 日本体育大

石塚　皓造　東大農農芸化学・渋谷区代々木初台町 701

小野　幹雄　東大理植

金井　弘夫　東大理植

木下　広野　星薬科大・三鷹市下連雀 305

木下　三郎　静岡大文理・静岡市緑町 29

相馬　研吾　東大理植

高木　嘉昌　東大農農芸化学坂口研究室

高宮　篤　東大理植

館岡　亜緒　静岡県三島市国立遺伝学研究所

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浜谷　稔夫　東大農

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根ケ山和弘	山口県宇部市宇部県立宇部高校
藤田　哲夫	広島市皆実町 3 広島大教養生物
松田　修	愛媛県伊予郡松前町横田 365
丸山　巌	松江市乃木県立農科大附属高校
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小野　林	福岡市大坪町九大教養生物
生野喜和人	（旧姓田崎）大分県大分郡庄内町大学畑田
新　敏夫	鹿児島市鴨池町 201 鹿児島大文理生物
吉田　忠生	九大農水産植物

小倉 謙

小 倉 謙 教 授 肖 像 画

安 達 真 太 郎 氏 筆

Portrait of Professor Yudzuru Ogura

By Shintaro Adachi, 1956

Professor Yudzuru Ogura and his Works

by Shunji WATARI*

亘理俊次*: 小倉謙教授と業績

On June 26, 1955, Professor Yudzuru Ogura celebrated his sixtieth birthday and retired on March 31, 1956, after his nearly fourty years of long sincere and meritorious service to the University of Tokyo. For his lucid contributions, the University appointed him to emeritus professor, the Botanical Society of Japan dicided to honour him by the issue of this special number, and his direct associates and intimate acquaintances had a commemorative assembly on an evening in April.

Prof. Ogura was born in 1895 at Sendai City, passed there his childhood and youth, and educated at the Second Highschool. In 1916, he entered the College of Science, Imperial University of Tokyo and studied at the Botanical Institute. In the final course, he specialized in plant morphology under the direction of Prof. Kenjiro Fujii. Immediately after graduating from the University in 1919, he was appointed as lecturer of the University, received the degree of Doctor of Science in 1927, promoted to assistant professor in the same year, and, in 1938, to professor, a position which he was continued to occupy until his retirement.

In Feb. 1928 he started for Europe as a researcher sent from the Department of Education. After staying for several months at the British Museum, London, and Botany School, Cambridge, where he was acquainted with Dr. D. H. Scott and Prof. A. C. Seward, he went to Munich when Prof. Karl von Goebel was the Director of the Botanical Institute, and returned to Japan via United States in April, 1930. He visited Europe again in 1954 and attended the Eighth International Botanical Congress at Paris as a representative of Japan.

During these years, he contributed significantly to the University as a councillor and as the director of the Botanic Gardens. He is also in service, as a commettee or councillor, to the Department of Education, the Science Council of Japan, the Natural Science Museum, and other organizations. To the Botanical Society of Japan, he devoted especially for long years as a general secretary and councillor, and in the last ten years (1946-1955) he was continuously elected President. In every occasion, Prof. Ogura's attainment, sound judgement, unselfishness, and ardent service commanded the respect of all who came in contact with him.

Contributions to the morphology and anatomy of pteridophytes.

Immediately after the publishment of his earliest work on the growth in thickness of trees (1920), Prof. Ogura turned into the investigation on the pteridophytes,

* Botanical Institute, Faculty of Science, University of Tokyo. 東京大学理学部植物学教室

commencing from an extensive historical review on their general structure (1921b). His first contribution in this field is "Gaps of stele in Polypodiaceae" (1921a) based upon more than thirty species of the family. His conclusion on the difference between the dictyostely and solenostely in this family is that it is irrespective of the length of the internode but chiefly owes to the length of gaps in the respective species.

In the meantime, his attention on many serious disagreements in the anatomy of the tree-ferns held by previous investigators led Prof. Ogura into his extensive studies on Japanese species of *Cyathea, Alsophila,* and *Cibotium* (1925 a–c, 1926 a–d), and also on a polypodiaceaous tree-fern *Diplazium esculentum* (1927 a). On the basis of these studies together with some additional observations he worked out a voluminous paper entitled "Comparative anatomy of the Japanese Cyatheaceae" by which he was granted a degree of Doctor of Science (1927 b). Among various features here described or discussed, a special importance seems to lie in an admirable explanation on the complicated stelar system of the stem which is mostly associated with the medullary bundles and also frequently with the cortical ones as well as of the petiole. He traced the origin of medullary bundles in young plants and found that they arised independently in the pith, but in the rare cases of the young form, they appeared from the innerside of the stele, suggesting that they might be derived from those of the polycyclic stele. He proposed here the names "Cyathean dictyostele" and "Alsophilan dictyostele", the latter differing from the former in the presence of cortical bundles which originate independently in the cortex. He further pointed out that the important structural features by which tribes of the family, i. e. Thyrsopterideae, Dicksoniae, and Cyatheae can be distinguished, are the types of stelar system in the stem, absence or presence of the sclerenchymatous sheath around the stele, and the characteristic arrangement of petiolar bundles.

On the way of his return to Japan from Europe, Prof. Ogura had an opportunity at Glenwood of the Island of Hawaii to collect three species of *Cibotium, C. Chamissoi, C. Menziesii* and a new species *C. hawaiense* Nakai et Ogura. He found (1930 b) that features of these species, especially an absence of the sclerenchymatous sheath around the dictyostele, are quite identical with the case of a well-known species with the creeping habit, *C. Barometz,* and he, hence, established a new tribe Cibotieae which stands very closely with the tribe Dicksonieae to which this genus had formerly been placed.

During his stay in Munich, 1929, Prof. Ogura studied the morphological and anatomical studies of *Polypodium* in a wider sense based on the specimen of the Munich University, and its results were published in 1935. Here he described in detail the structure of hairs of the petioles, nerves and sporangia, and discussed the variablity of the genus. He added further two important papers. In a detailed study on the external and internal features of *Oleandra Wallichii* and other several species (1938 b), he concluded that the root-like organ which springs out of the

stem exogenously is to be understood as "rhizophore", and that the genus should be placed in an isolate phyletic position, an independent family, Oleandraceae. In another paper (1939), on the basis of a particular vascular course, especially of the medullary bundles, he established Acrostichum-type based on *Acrostichum speciosum*, which is slightly different from the Cyathean-type.

Building upon his own studies and on the basis of an accumulation of vast knowledge on the pteridophytes, he published in 1938, his comprehensive work "Anatomie der Vegetationsorgane der Pteridophyten" as a part of "Handbuch der Pflanzenanatomie". Here he established firmly his excellent stelar theory and published many precious conceptions, together with detailed descriptions on living and fossil members. To his great contributions to the anatomical works on pteridophytes, the Japan Academy honoured him, in 1946, with the bestowal of the Japan Academy Prize.

Contributions to the palaeobotany.

Since an account of the Cretaceous plants from Hokkaido was published by Stopes and Fujii in the Philosophical Transactions of the Royal Society of London (1910), more than fifteen years have elapsed without any noteworthy works on the petrified fossils, until Prof. Ogura published successively his papers since 1927. His work in this field was commenced from a study on the Mesozoic tree-ferns from Japan and Korea (1927 c), wherein he proposed three new species, i. e. *Cyathocaulis naktongensis*, *Cibotiocaulis Tateiwae*, and *Cyathorachis Fujiiana*, the latter being the very finely preserved petiole. The former two show the Cyathean-type of the dictyostely but differ from each other in the feature of the leaf-scar as well as in the arrangement of leaf traces. He studied many further specimens obtained by himself at Korea (1941 a) and several important features have been ascertained or added to these species. He pointed out here that the best feature in distinguishing these two species and *C. Yabei* (1941) from the Tertiary of Mindoro, Philippine Is., lies in the structure of medullary meristeles and their sclerenchymatous sheath. From these studies, he came to the conclusion that the differences between *Cyathocaulis* and *Cibotiocaulis* are too small to place them in different genera and united the latter to *Cyathocaulis*. He also described two fossils related to the Cyatheaceae, i. e., *Protocyathea Tokunagai* (1931) and *Cibotium iwatense* (1933) from the Upper Cretaceous of Japan, the latter being similar in the absence of medullary meristeles and of the sclerenchymatous sheath around the vascular bundles (cf. 1930 b).

In 1928, Prof. Ogura spent many days at the British Museum for the examination of sections collected by Prof. Fujii and himself from the Cretaceous of Hokkaido. As soon as he returned to Japan, he published a paper (1930 a) in which he proposed *Yezopteris polycycloides*, a fern stem which is very close to the Cyatheaceae, *Solenosteleopteris loxsomoides* resembling *Loxsoma* of the Davalliae, a cycadean petiole *Cycadeoidea petiolata*, and a small and slender cycadean shoot *Cycadeoidella*

japonica. Also he emended here Stopes and Fujii's description on *Cunninghamio-strobus* basing upon two new specimens. This work was soon followed by the second contribution (1932 a) which owes largely to the specimens obtained by himself in his two visits to Hokkaido during 1930 and 1931. Here he added *Cycadangium compactum* representing a new type of the cycadean microsporophyll, *Stachycarpites projectus* on the basis of seeds having an affinity to *Podocarpus*, especially to *Stachy-carpus*, *Piceophyllum simplex* a leaf close to *Picea* or *Larix*, a three-leaved pine *Pinus flabellifolia*, a five-leaved pine *Pinus pseudostrobifolia*, *Sciadopytis cretacea* closely resembling to our living umbrella pine, and *Yubaria invaginata* which is a dicotyledonous petiole with uncertain affinity.

In the same year, Prof. Ogura described an extinct evergreen oak, *Quercinium hobashiraishi* (1932 b) by studying a huge silicified trunk, a famous natural monument "hobashiraishi" lying on the beach at Najima near Fukuoka City. It is to be remembered as the first sure record of the dicotyledonous wood from the Tertiary of Japan. He also found at the same place another noteworthy species, *Phyllanthi-nium pseudohobashiraishi*, an euphorbiaceous wood resembling especially to *Bishoffia* (1932 c). His third dicotyledonous wood *Castanopsis Makinoi* was published in 1949.

In the later year (1944), Prof. Ogura proposed five new species of coniferous woods, i. e. two *Araucarioxylons*, *A. kiiense* and *A. jeholense*, *Taxodioxylon compres-sum*, *Cedroxylon simplex*, and *Pinuxylon microporosum*. Also he added here a noteworthy knowledge to *Xenoxylon latiporosum*, a gymnospermous wood with a doubtful affinity, as well as a summarized information on Japanese *Taxodioxylon*. Although his interest on *Xenoxylon latiporosum* was shown already as early as 1931 when he briefly noted its occurrence from Korea, he described first in this account his careful observation on the thin-walled septa in the lumen of tracheids which are tyloses of tracheids derived from the adjacent ray cells. Several years later, nume-rous examples including a considerable number of erect stumps of this species were found from the Jurassic of Isikawa Prefecture, Japan (1951). He succeeded to observe the structure of a fairly well-preserved pith in one of them, on which he communica-ted at the Eighth International Botanical Congress held at Paris in 1954. Though the report in detail is not yet published, it is to be remarked here that the pith of *Xenoxylon* should be regarded as an ordinary coniferous type in its structure.

He studied also a large silicified stem base covered with crowded roots, sitting as ornament in the famous Kenroku park at Kanazawa City. Although its exact origin was uncertain, he identified it as a new species of *Palmoxylon*, *P. Maedae*, belonging to Stenzel's Cordata group of the Corypha type (1952 a). Soon after, how-ever, he found another closely related species, *P. kagaense* (1955 b), among the gravels at a river-bed of the same district. It was probably derived from a green-tuff of the Miocene, and the finding of this species is particularly important since it immediately suggests, as he himself thought, a possible occurrence of *P. Maedae* at a certain locality of the same district.

On the other hand, the sole but very important contribution on the Palaeozoic fossil was published in 1948, in proposing a new name *Pecopteris samaropsis*, a seed-bearing Pteridosperm, from the Upper Permian of Penhisu coal fields, Manchuria. It was described on the basis of an excellent specimen with organic connection of frond and seed which have been known until that time as *Pecopteris arborescens* and *Samaropsis affinis* respectively.

Contributions to the morphology and anatomy of higher plants.

Prof. Ogura's earliest work "Some observations on the growth in thickness of trees" (1920) was carried out under the direction of Prof. Kenjiro Fujii during his final course of student (1919–1920). Of the woods of *Cryptomeria* and other several conifers and angiosperms, he measured extensively the width of annual rings and dimension of xylem elements in variable ages and heights, and he found several important correlations among them, some of them being nearly confirmative of Sanio's law, while his conclusion on the correlation between climatic factors, especially the amount of precipitation and the growth in thickness is rather negative.

After a fairly long interval during years when Prof. Ogura was busy in the study on the pteridophytes and Mesozoic fossils, he contributed again many important works in this field. In a paper dealt with several species of *Blahdia* (1937 b), he reported a new type of the branch abscission in *B. Sieboldii* and other species whose base of branch is especially widened, and when they disarticulate from the stem there leave large scars, on whose periphery vascular bundles are loosely arranged. On the basis of observations on the mangrove plants during his visit to the Micronesian Islands in summer of 1939, Prof. Ogura described (1940, 1942) two new tpyes of the aerial roots, "standing roots" (*Urandia ammui* and *Glochidion hongkongenense*) and "curving roots" (*Horsfieldia amklaal*). The first type resembles erect roots but differs in essentially similar structure with subterranean roots, and the second type is comparable with curved-knee roots but differs in the simpler shape and in the absence of abnormal thickenings. In his study on the branching habit in the genus *Phyllanthus* (1943), he showed that *Ph. Matsumurae* represents the simplest type in which only a kind of branch is present, whereas are seen two types of branches, i.e. long and short ones, in *Ph. Urinaria* and *Ph. flexuosus*, the latter differing from the former in more acceralated difference between both branch types.

During these years, Prof. Ogura also accomplished an extensive study on the dimension of bast fibers (1940 b), in enlisting more than 350 species including some pteridophytes and gymnosperms as well as many dicotyledons and monocotyledons. In an observation on the formation of root tubers in *Ipomoea Batatus* (1945), he showed that the tubers are formed from the normal roots by the abnormal thickening due to the activity of cambia around the vessels.

Another series of interesting works published in recent years is observations

in the morphology and anatomy of subterranean organ of some monocotyledons. In a study on the Liliaceae (1952 b, c) Prof. Ogura was especially aware of *Erythronium japonicum* whose club-shaped subterranean organ is provided with a curious appendage consisting of a certain number of segments. In going carefull comparisons with some allied genera such as *Tulipa*, *Gagea* and *Lloydia*, he came to a recognition that the club-shaped organ in *Erythronium* is a bulb caused by the fusion of two or three sheaths and its basal part is bulged out annually as a small segment, which remains as the peculiar appendage. He also studied *Allium monanthum* (1955) whose bulb consists of a few enclosed scale and includes usually one adventitious bulblet. Here he showed that the bulblet concerns in a peculiar way to the vegetative propagation, that is, it germinates in the next spring into a long stolon whose tip contains, at the base of leaf, usually two bulblets and one of them, the adventitious one, repeats the formation of stolon in the subsequent spring.

In another work (1953), Prof. Ogura treated many species of the Ophyrydinae of the Orchidaceae. In every plants, besides normal roots, there are one or two special subterranean organs which are provided with a bud in its certain part. On the basis of a precise anatomical study he recognized that the proximal portion is a combined system of a central cauline axis and peripheral root traces, while the distal portion is a combined system of some roots. He concluded that the proximal portion may be considered as a rhizophore which carries the distal portion of a radical character.

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Some Thoughts on the Structure of Bamboo Leaves

by C. R. METCALFE*

1. Introduction

In the course of a general survey of the comparative anatomy of the vegetative organs of the Monocotyledons, which is now in progress at the Jodrell Laboratory, at Kew, I have devoted considerable attention to the Gramineae. It is becoming increasingly recognized by students of grass taxonomy that certain microscopical characters visible in the leaf epidermis, and in transverse sections through the lamina, are of considerable importance in the basic classification of the Gramineae. Other microscopical characters of the vegetative organs are also valuable if used judiciously, for identifying species when the genus has already been established by traditional macroscopical characters. This has been fully confirmed by the work at Kew, and indeed, taking a broad view of the Gramineae, it can be seen that there is an anatomical pattern which roughly corresponds to some of the major groups into which the grasses are divided for purposes of classification.

Although much attention has now been devoted by various authors, to a wide range of grasses, it is a notable fact that comparatively little attention has been devoted to the bamboos, although some very useful contributions have been made. Thus we have the detailed work of Ohki (3, 4) on the spodograms of the leaves of the Bambusaceae, in which the epidermal characters of a number of species of some 10 genera have been described. At a much earlier date there was the work by Brandis (1), whose paper has become a classic, and, more recently Prat (6) has referred to the structure of bamboo leaves as part of his well known contribution to the anatomy of the epidermis of grass leaves. Page (5) has reviewed the leaf structure of *Streptochaeta* in relation to that of the bamboos, and certain African bomboos have only recently received attention from Jacques-Félix (2). These selected references by no means exhaust the bibliography, but they will serve as a background for the present discussion.

2. Transverse section of the lamina

(i) Structure of the mesophyll.

It is familiar to most students of grass anatomy that the structure of the as-

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* Jodrell Laboratory, Royal Botanic Gardens, Kew, Great Britain
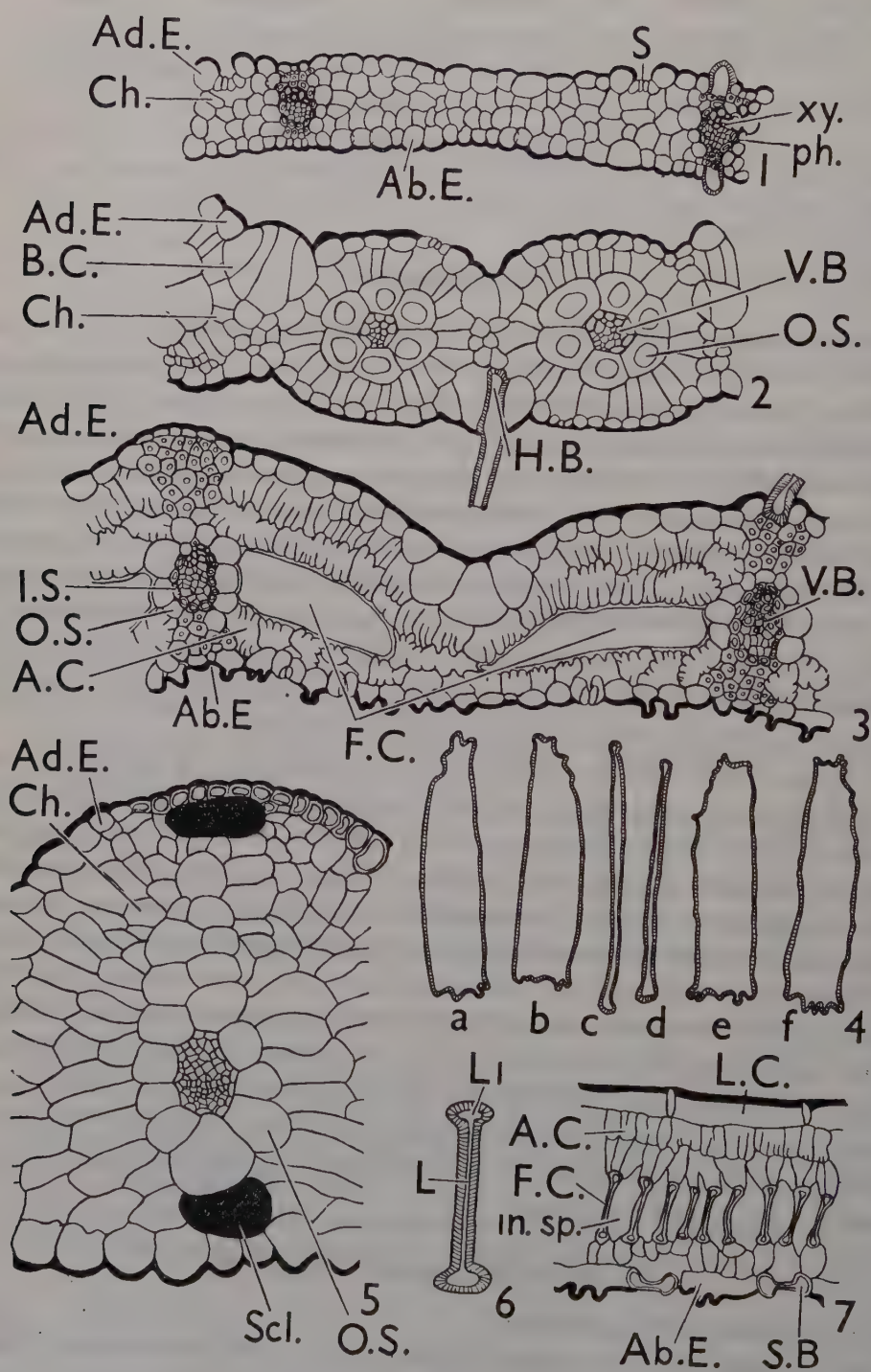
similatory tissue in the mesophyll of most panicoid grasses is radiate, in the sense that the cells of which it is composed are arranged in a radial manner around each of the vascular bundles. In the festucoid grasses no indication of the radiate arrangement of the mesophyll around the vascular bundles is to be seen. As more and more grasses are examined, however, it is found that there is no clear cut line of demarcation between these 2 types of mesophyll in grass leaves, since there are some in which a vague, or incompletely radiate arrangement of the mesophyll is to be seen. Furthermore one can distinguish grasses with relatively short assimilatory cells arranged in a very conspicuously radiate manner, e. g. *Panicum capillare* Linn. (Fig. I, 2) from those in which the radiating cells are long, narrow, and often form a somewhat spongy part of the mesophyll. This type of structure (Fig. I, 5) has been noted particularly in certain species of *Isachne* and allied genera, and it may for convenience be termed the *Isachne* type of mesophyll. *Melica uniflora* Retz. (Fig. I, 1) affords a good example of a grass with the non-radiate type of mesophyll. For our present purpose it should be noted that the Bambuseae, so far as I am aware without exception, exhibit the non-radiate arrangement of the mesophyll.

Fig. I. 1. *Melica uniflora*. T.S. lamina with festucoid mesophyll, the chlorenchyma (Ch.) not being arranged radiately around the vascular bundles. ×220.

2. *Panicum capillare*. T. S. lamina with panicoid mesophyll, the chlorenchyma being arranged radiately around the vascular bundles. ×220.

3. *Arundinaria auricoma*. T. S. lamina showing the type of mesophyll characteristic of bamboos, with chlorenchyma composed of arm-cells (A. C.) and translucent fusoid cells (F.C.) × 220.

4. *Dinochloa m'clellandii*. a, b, e & f fusoid cells from a macerated leaf lying on their sides, i. e. more or less as they appear in transverse sections of the leaf at right angles to its long axis. c & d fusoid cells, from a macerated leaf, standing on their edges, i. e. as they would appear in a paradermal section. × 220.

5. *Isachne kunthiana*. T. S. of a small portion of the lamina passing through a vascular bundle, showing the chlorenchyma arranged in a more or less radiating manner around the vascular bundle. The assimilatory cells are often narrower and more elongated than those shown in the figure. This type of mesophyll, which is very spongy, and of which it is difficult to prepare sections, is highly characteristic of *Isachne* and allied genera. × 300.

6. *Arundinaria auricoma*. Reconstruction of a fusoid cell as seen in a T.S. of the lamina parallel to the long axis of the leaf. These cells always appear to have contracted, so that the narrow lumen L is often invisible or is seen as a single line. The wider portion of the lumen at either end of the cell (L₁) can sometimes be seen more clearly. The collapsed condition of the fusoid cells has led to difficulty in interpreting their structure.

7. *Arundinaria auricoma*. Lamina parallel to the long axis of the leaf, showing long cells (L.C.) alternating with short cells, of the adaxial epidermis; chlorenchyma composed of arm-cells (A.C.); a single row of fusoid cells (F.C.), separated from one another by intercellular spaces (in. sp.). Two dumb-bell shaped silica bodies (S.B.) are to be seen in the abaxial epidermis (Ab.E.). × 220.

Ab.E., abaxial epidermis. A.C., arm-cells. Ad.E., adaxial epidermis, B.C., bulliform cells. Ch. chlorenchyma. F.C., fusoid cells. H.B., base of hair. I.S., inner bundle sheath. in. sp., intercellular space. L, lumen of cell. L₁, wide lumen at end of cell. O.S. outer bundle sheath. ph, phloem. S., stoma. S.B., silica body. Scl., sclerenchyma. Xy., xylem.

Another important character in the mesophyll of grasses is that the assimilatory cells are sometimes provided with thin invaginations of the cell walls extending to different depths in the lumina of the cells. Cells of this type can be conveniently referred to as arm-cells. This character is of considerable diagnostic value because of its restricted occurrence amongst the grasses as a whole, but it is noteworthy that arm-cells are especially characteristic of the Oryzeae and Bambuseae. The arm-cells of *Arundinaria auricoma* are to be seen in Fig. I, 3, A. C. There is, however, one important respect in which the leaves of most bamboos stand out in marked contrast to those of most grasses. I refer to the occurrence of conspicuous, translucent cells in the mesophyll, where they usually have a somewhat fusiform outline when observed in transverse sections of the lamina (Fig. I, 3, F.C.). Brandis (1) recognized that these structures are cells, and not intercellular spaces as had been maintained by certain investigators before his time, although even Brandis himself referred to them as "apparent cavities". Since these cells are usually examined in transverse sections of the lamina, where they are approximately fusiform in outline, they may be referred to for descriptive purposes as fusoid cells. It must be emphasized, however, that the fusoid appearance of the cells is misleading, for they have the form of narrow plates that appear to be fusoid only when they are viewed from one side, either in macerated material (Fig. I, 4, a,b,e,f,) or in transverse sections (Fig. I, 3, A.C.) of the lamina, the long axes of the cells lying at right angles to the long axis of the lamina itself. The lumina of these plate-like cells (Fig. I, 6, L) are very narrow, and it is often very difficult to see them in sections of the leaf parallel with its long axis. Indeed the cells are so thin that they could at first sight be mistaken for cell walls. At the ends of the cells that respectively lie nearest to the adaxial and abaxial surfaces of the leaf, the lumina are slightly wider and more clearly visible (Fig. I, 6, L1 & 7, F.C.). The appearance of the cells in paradermal sections of the leaf is shown in Fig. I, 4, c–d. Sections parallel to the long axis of the leaf reveal that there are intercellular spaces (Fig. I, 7, in. sp.) between adjacent fusoid cells. Although their development has not been followed in detail, it seems reasonable to suppose that, when first formed, the fusoid cells are in contact with one another, and that they become separated from one another and assume their characteristic and unusual shape during the ontogeny of the leaf. These facts show conclusively that Brandis (1) was correct when he interpreted the translucent areas that are visible in transverse sections of the lamina as cells rather than cavities, and in this he has been followed much more recently by Page (5). It should, however, be noted that Jacques–Félix (2), writing much more recently than Page, refers to the cells as if they were cavities. Fusoid cells are especially characteristic of the mesophyll of bamboos as distinct from other grasses, although they vary considerably in size in different species and genera. In some bamboos, especially in *Phyllostachys*, they occur sporadically, or are apparently absent. None were to be seen in material of *Phyllostachys castillonis*, *P. heterocycla*, and *P. nigra*

grown at Kew, although they were found sporadically in *P. bambusoides*, *P. reticulata*, *P. ruscifolia* and *P. viridi-glaucescens*. Fusoid cells were observed in representatives of some 20 other genera of bamboos that were examined, and they were especially large in *Atractocarpa olyraefolia* and *Puelia ciliata*. It should be noted that fusoid cells have also been seen at Kew in species of *Neurolepis*, *Olyra*, *Pariana*, *Pharus*, *Streptochaeta* and *Streptogyne*, a fact which suggests that there may be affinities between these genera and the bamboos. Somewhat similar cells, which are, however, more nearly sausage-shaped, occur in *Leptaspis*.

(ii) Vascular structure of the midrib.

Transverse sections through the lamina of grasses, taken at a standard level about half way between the apex of the lamina and its base, often show a keel or midrib in a median position. The midrib varies from being scarcely perceptible to very conspicuous in different species, and within each species the appearance of the midrib varies according to the exact level at which the section is taken. The number of vascular bundles in the midrib varies from 1 to 24 or more, the number of bundles being proportional to the area of the transverse section of the midrib. In most grasses with large midribs the vascular bundles are arranged in an arc not far from the abaxial surface, and the bundles themselves are often very unequal in size, or of several distinct sizes. When the midrib is small it generally contains a single median vascular bundle. When one turns to certain taxonomic groups of grasses, however, the simple arc of bundles is found to be replaced by a more complex vascular system. This applies, for example, in certain of the Oryzeae, such as *Zizania latifolia* Turcz., where, in addition to an abaxial arc of bundles, there is a row of about 5 bundles just below the flat adaxial surface of the midrib, and a few others in a median vertical plane. In this species there are also large intercellular spaces in the ground tissue, which are no doubt a specialization related to its aquatic environment.

The midrib of *Zizania* is interesting because it is in some ways intermediate between the midrib structure exhibited by most grasses on the one hand and the more complex types characteristic of a large majority of the bamboos. It may be noted in passing, however, that *Merostachys riedeliana* differs from the other bamboos that I have examined in having no well defined midrib, but this would appear to be exceptional amongst the bamboos as a whole. The appearance of transverse sections of a selection of bamboo midribs cut midway between the apices and bases of the lamina is shown in Fig. II, 3–11. In Fig. II 1A–1C and 2A–2B transverse sections taken at successively lower levels through single midribs of *Dendrocalamus brandisii* and *Shibataea Kumasasa* respectively are shown. It is clear from these diagrams that although the vascular system of the midrib is more complex towards the base than towards the apex of leaf, the fact remains that the midribs of most of the bamboos that I have examined have more complex systems of bundles than is characteristic of other grasses, and the bundles are supported by comparatively
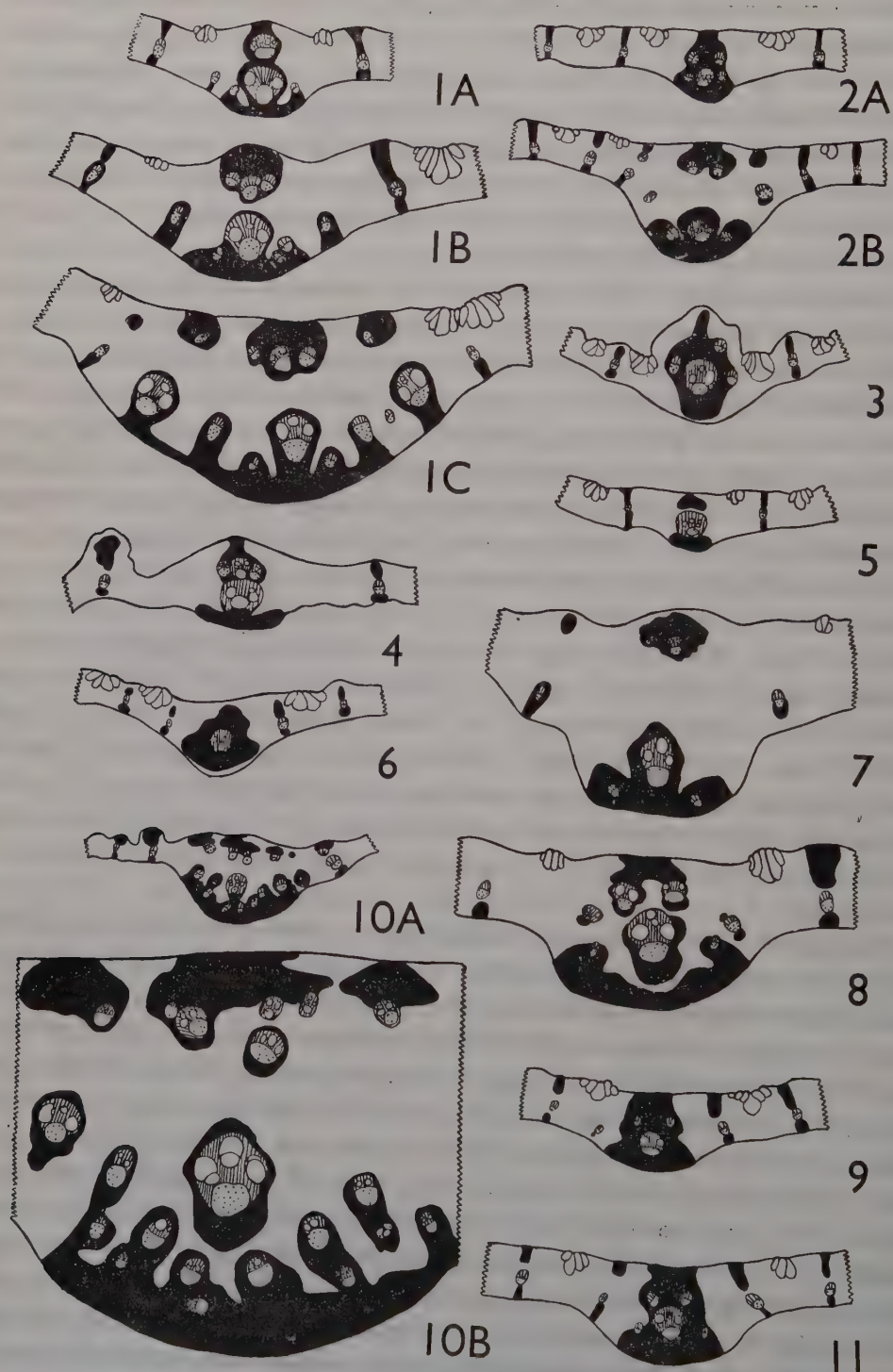
elaborate systems of sclerenchyma. It should be noted, however, that in certain bamboos e g. *Arundinaria murielae* (Fig. II,5) and *Chusquea abietifolia* (Fig. II,6) the vascular system is comparatively simple, but the bundles are well supported by sclerenchyma. In most of the species, however, it can be seen that the midrib bundles consist essentially of an adaxial and an abaxial series, all of the bundles being embedded in, or associated with, sclerenchyma. The number of bundles in the adaxial and the abaxial series respectively varies from one species to another. Furthermore the adaxial and abaxial series are, in some species, embedded in, and united by, one more or less continuous mass of sclerenchyma. In other species the adaxial and abaxial series of bundles are separated from one another by thin-walled ground tissue. Differences of this kind can be used to some extent for specific diagnostic purposes.

It is interesting to note that a few grasses not regarded as members of the Bambuseae, exhibit a type of vascular structure in the midrib that recalls that of the bamboos. This applies for example to *Neurolepis nobilis* Pilger and *Streptogyne gerontogaea* Hook. f., whilst similar but less complex types of structure have been noted in *Olyra latifolius* L., *Pariana bicolor* Tutin, *P. campestris* Aubl., *P. vulgaris* Tutin and *Pharus latifolius* L.

(iii) Bundle sheath.

It is not possible here to enter into a full discussion of the types of bundle sheaths that occur in the bamboos. It must suffice to say that, like previous investigators, I have found the sheaths always to be double, the cells of the outer sheaths being thinner-walled and more parenchymatous than those of the inner sheaths. This fact is of interest because the double type of bundle sheath is usually regarded as a festucoid character.

3. Abaxial epidermis

(i) Short cells and silica bodies.

As with other grasses, it is necessary when studying the epidermis of bamboos,

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Fig. II. 1A—1C. *Dendrocalamus brandisii.* T.S. midrib at 3 successively lower levels to show the range of structure in an individual leaf. × 60.

2A—2B. *Shibataea Kumasasa.* T.S. midrib at 2 successively lower levels to show the range of structure in a single leaf. At a higher level in the leaf than that illustrated in 2A the midrib is marked by only a single vascular bundle.

3—11. T.S. midribs of bamboos cut in the position that was adopted as a standard for comparative purposes, i.e. about half way between the apex and base of the lamina. All except 10a × 60.

3. *Arthrostylidium capillifolium.*	4. *Chusquea tenella.*	5. *Arundinaria murielae.*
6. *Chusquea abietifolia.*	7. *Atractocarpa olyraefolia.*	8. *Oxytenanthera abyssinica.*
9. *Phyllostachys bambusoides.*		
10. *Gigantochloa ligulata.*	10A ×12. 10B A similar, but not identical, section × 60.	
11. *Phyllostachys viridi-glaucescens.*		

Solid black, sclerenchyma. Dotted areas, phloem. Vertical lines, xylem. The clusters of large cells in the adaxial epidermis are bulliform cells.

to distinguish the costal zones above the veins from the intercostal zones between them. In the intercostal zones themselves there are longitudinal strips with and without stomata respectively, and the epidermis, both above and between the veins, is made up of "long" and "short" cells respectively, and dermal appendages of the various types that occur in grasses are also present. The short cells of bamboo leaves, like those of other grasses, can be divided into those which contain silica bodies and those which do not. Short cells between the veins are nearly always in pairs, but occasionally solitary, whilst those over the veins may be in pairs, short rows along the veins, or in much longer but similar rows. Of the 45 species of bamboos belonging to 24 genera that I have examined it has been found that some species are characterized by having the short cells that lie over the veins in pairs, whilst in the other species they are in rows, and, in a third group of species there is a mixture of both types of distribution. Silica bodies above the veins are predominently saddle-shaped, and indeed very few of the bamboos that I have examined are entirely devoid of saddle-shaped silica bodies. In some bamboos the saddle-shaped bodies are tall in a direction that lies transversely to the long axis of the leaf. Bodies of this kind could be classified as being of the *Oryza* type. In some species, bodies that are cross-shaped, dumb-bell shaped, or intermediate between those 2 types occur over the veins, where, they are sometimes intermixed with silica bodies of the dominant saddle-shaped type. In a few species the silica bodies are exclusively cross-shaped and dumb-bell shaped.

Turning now to the short cells between the veins, it may first be noted that there are some species from which intercostal short cells are absent. Where intercostal short cells containing silica bodies are present, however, it is usual to find that, like those over the veins the silica bodies are mostly saddle-shaped, but in some bamboos they are tall and narrow, and may be oval, rounded, or crenate in outline.

(ii) Micro-hairs.

Apart from the short cells and the silica bodies, it can also be seen from epidermal preparations that micro-hairs, i.e. small hairs that are generally 2-celled, are almost invariably present on bamboo leaves, although they are sometimes difficult to find. Of the 45 species that I have examined there were only 2, *Atractocarpa olyraefolia* and *Puelia ciliata*, in which micro-hairs were not detected in the available material on the abaxial surface of the leaf. The hairs most frequently occur in the intercostal zones in longitudinal strips from which stomata are absent. In a few species they occur almost amongst the stomata. Although the sizes, shapes and frequencies of the micro-hairs vary from species to species, they are mainly of one basic type in which both cells are of more or less equal length, the distal cell being of approximately uniform diameter throughout its length, or sometimes tapering slightly towards the apex. In some species, however, particularly in *Arundinaria*, the basal cell is appreciably longer than the distal cell. *Guaduella oblonga* is distinguishable

from the other bamboos that I have examined in having micro-hairs of which each consists of a long basal cell and a distal part which in uniseriate or partly biseriate. The occurrence of micro-hairs in bamboos is of interest because, amongst other grasses their presence is regarded as a panicoid character. They appear to be of restricted diagnostic value amongst the bamboos themselves.

(iii) Long cells and papillae.

The long cells throughout the bamboos are remarkably uniform in type, most of them having sinuous walls. Those of the intercostal zones generally have rather thinner walls, and usually wider, lumina, than those over the veins. The outer walls of the long cells are usually abundantly papillose, the papillae being variable in shape and in the extent to which they are cutinized. There are some bamboos in which papillae are infrequent. Often, besides the outwardly directed papillae, there are others that are bent over the stomata and serve for their protection. The number of papillae overarching the stomata in this way varies from species to species, and from some species they appear to be absent. Sometimes papillae occur around the stomata although they are absent elsewhere on the long cells; in other species, although papillae are present, few of them overarch the stomata. Papillae are specially abundant on long cells in the intercostal zones, but they sometimes occur over the veins as well. Differences in the frequency and distribution of papillae on the long cells appear to be of specific diagnostic value, but are of little diagnostic value at the genus level.

4. Taxonomic conclusions

The anatomical observations recorded in this article represent only a small proportion of those which I have made on the structure of bamboos during the past few years. They do, however, point to certain conclusions. Firstly it may be noted that, in the bamboos, we find a combination of anatomical characters that is unusual for the grasses as a whole, and some characters that are peculiar to the bamboos and only a few genera that are not generally regarded as bamboos in existing systems of classification. For example the bamboos exhibit not-radiate assimilatory tissue and double sheaths, two characteristics of festucoid grasses. On the other hand the occurrence of micro-hairs, of cross and saddle-shaped silica bodies, and of short cells in longitudinal rows above the veins, are panicoid characters. There is also a tendency for the saddle-shaped silica bodies to be of the *Oryza* type in certain species. The fact that the assimilatory tissue usually consists of arm-cells is another character that the bamboos share with the Oryzeae. Taken together, these facts show that certain of the characters that occur in combination in the bamboos have become separated in other groups of grasses, which suggests that the bamboos may be more primitive than other grasses. It should be noted, however, that the bamboos clearly stand out amongst the grasses as a distinct group, apart from a few isolated genera, in having the highly characteristic fusoid

cells in their mesophyll, and in the complex vascular structure and massive deve-
lopment of sclerenchyma in their midribs.

Another important point is that, although the bamboos exhibit combinations
of characters that appear to be diagnostic for the species in which they are ex-
emplified, there are no clear anatomical lines of demarcation between the genera.
Indeed to the anatomist the bamboos appear to consist of numerous species that
could be interpreted as belonging to only one, or at most only a few, genera.
Further work on the structure of the vegetative organs, and of the flowers and
fruits, will be necessary before the taxonomy of the bamboos can be more fully
understood.

Species examined

Arthrostylidium capillifolium Griseb., *A. pubescens* Rupr., *Atractocarpa olyraefolia*
Franch., *Arundinaria auricoma* Mitford, *A. fastuosa* Mak., *A. fortuni* A. & C. Riv.,
A. graminea Mak., *A. japonica* Sieb. & Zucc. (*Sasa japonica* Mak.), *A. marmorea*
Mak., *A. murielae* Gamble, *A. nitida* Mitford, *A. pumila* Mitford, *A. simonii* A. & C.
Riv., *A. tessellata* Munro, *A. vagans* Gamble, *Bambusa nana* Roxb., *B. vulgaris*
Schrad., *Cephalostachyum capitatum* Munro, *Chusquea abietifolia* Griseb., *C. tenella*
Nees, *Dendrocalamus brandisii* Kurz, *D. giganteus* Munro, *Dinochloa m'clellandii*
Kurz, *Gigantochloa ligulata* Gamble, *Greslania rivularis* Bal.. *Guadua paniculata*
Munro, *Guaduella oblonga* Hutch., *Melocanna bambusoides* Trin., *Merostachys*
riedeliana Rupr., *Nastus capitatus* Munro, *N. elegantissimus* (Hassk.) Holttum
(*Chloothamnus elegantissimus* (Hassk.) Henr. and *Schizostachyum chilianthum* Kurz),
Ochlandra setigera Gamble, *Oreobambus buchwaldii* K. Schum., *Oxytenanthera*
abyssinica (A. Rich.) Munro, *Phyllostachys bambusoides* Sieb. & Zucc., *P. castillonis*
Mitford, *P. heterocycla* Matsum., *P. nigra* Munro, *P. reticulata* C. Koch, *P. viridi-*
glaucescens A. & C. Riv., *Pseudostachyum polymorphum* Munro, *Puelia ciliata* Franch.,
Shibataea Kumasasa Mak., *Thyrsostachys oliveri* Gamble.

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On the Phylogeny of the Stele

by W. Zimmermann*

1. Methods

Our aim is to demonstrate the actual evolution of the stele. We wish to exhibit the phylogenetic process of life as it really took place in bygone time (viz. the historical process in the past and not an abstract performance in our mind). We thus search for the ancestral stages, for instance of the first steles in early land plants, and on the other hand for the events that lead from those early stages to the recent types of the stele.

There is a vast divergence of views about the phylogeny of the stele, caused by differences in methods. It results mainly from the varying interpretation of the word phylogeny that should be applied only to the actual, " real " (naturwirklichen) evolution process and not to an only intellectual linkage of the organisms. Up till now, for historical reasons, the notion phylogeny or evolution is often used in confusion or combination of the natural process with the human mental process. But in an exact methodology the word phylogeny should not be employed for speculative deduction[1] of a recent organ or organism from another recent organ or organism nor for derivation of ontogenetically " complete " steles from each other. Both these deductions contain partially right perceptions though mixed up with wrong conclusions.

For instance, a recent organism may represent some primitive features; but this does not justify to infer further: it is to be regarded as primitive in general, and it would be false to derive from such an organism any phylogenetically earlier organisms.

Equally, a series of ontogenetically " complete " steles may serve to exhibit the general course of phylogeny. But such isolated stages can not manifest the living

process of stele development which is more versatile and, for all, more complicated. In fact this process is what I called a "hologeny" (Fig. 1), namely a continuous series of ontogenies that begins with the ontogeny of the ancestors.

To seize the actual phylogenetic development of the stele we have to be conscious of the hologenetic relation. Moreover, we have to bear in mind: a phylogenetical change starts with changes of the genotype: this genotypic change induces an alteration and wandering of the determining impulses in the embryonal tissue; in consequence, the embryonal development shows an altered differentiation with regard to shape, position and sequence of organ formation.

With reference to the stele that means: also the ancestors developed an "initial tissue" (procambium, protophloem and protoxylem) near the growing point. In accordance with the physiological development these initial stages subsequently originated the ontogenetically "complete" stele which is characterized by typical cross sections, as represented in Fig. 4.

In the descendants, under the influence of the changed genotype, the determining factors of the embryonal tissue begin to act in the initial stages, and particularly in the later stages. The growing point is undergoing a kind of new-cast, whereby a "shifting" of the tissue can take place; this process is marked by a changed habitus, by a changed local arrangement, and by a changed timely sequence of the

Fig. 1.　Hologeny of the stele.

"complete" tissue.

II. The original structure of the stele

According to prevailing opinion the original type of the stele is the protostele with central protoxylem. This type is repre-
sented by the telomes and mesomes of
the *Rhyniaceae* (*Rhynia*[2]) and *Horneo-*
phyton) and some *Primofilices.* The
protostelic telomes are characterized by
a strong radial symmetry. The tissues
are arranged in layers of concentrical
cylinders: The central protoxylem is
surrounded by the cylindrical metaxylem
(as in *Rhynia major* and *Horneophyton*,
see Fig. 2), then by a cylinder of phloem
(or a precursor of phloem) and finally by
a cortical parenchyma and the epidermis.
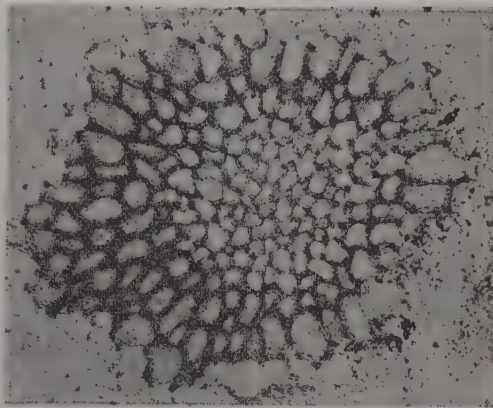
Fig. 2.   Protostele of *Rhynia major.*

Ontogenetically we discern, already in the vicinity of the growing point, the central procambial strand that gives rise to the first permanent tissue: the protoxylem. This arrangement indicates that here, as in later axes, the initial stele acts on the ontogenetically later tissue in the sense of an organizer ("Organisa-tor"). Especially in the formation of the metaxylem and secondary xylem the deter-mining influence of the protoxylem (or its meristematic precursor) is quite obvious.

Apparently this protostelic structure of archae-syntelomes in *Psilophyta* is the archetype of the different steles. This is to be proved in the same way as for the archae-syntelomes themselves (Fig. 3). Protosteles and archae-syntelomes dominate in the oldest land-plants. The primary protosteles dichotomise like the primary syntelomes.

III. The initial stage of derivated steles

The ontogenetically complete steles (Fig. 4) are marked by an extreme difference in appearance, while the initial stages show an astonishing resemblance.

In almost all cases we find a *cylinder of strand-initials* ("Initialen-Buendelrohr" Fig. 5A and 9B). That means, there appears at the beginning of vascular differen-tiation a cylinder of strands in the vicinity of the growing point. These strands represent an "open", non-anastomising system, at least in the axes of archaic stems. The same open structure prevails in all primitive organs (as well in leaves as in axes). The initial strands of this cylinder are forked into cauline bundles ("meriste-les") and leaf traces (Fig. 9B).

In these initial stages the differences between the various types of the stele are restricted mainly in two features:

1) The number of cauline bundles varies from 3 (e. g. *Sphenophyllum*) or 4 (*Stauropteris*) or 5 (*Lyginopteris*) to more than 100 (*Calamites*).

2) The leaf traces branch off in different directions (as indicated in Fig. 5A).

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2) *Rhynia* itself is found much later than the other genera, other members of *Rhyniaceae* ex-
isted already in the Silurian time.

Makrophylle □

Oberdevon
43　42　17　34 %

Hemi-Kormophyta ▥

Mitteldevon
16　8　24　40　12 %

Mikrophylle ▨

Unterdevon
38　22　21　19 %

Psilophyton ▦

Australisches Silur
80　20 %

Rhynia ■

Nordeurop. Silur
88　12 %
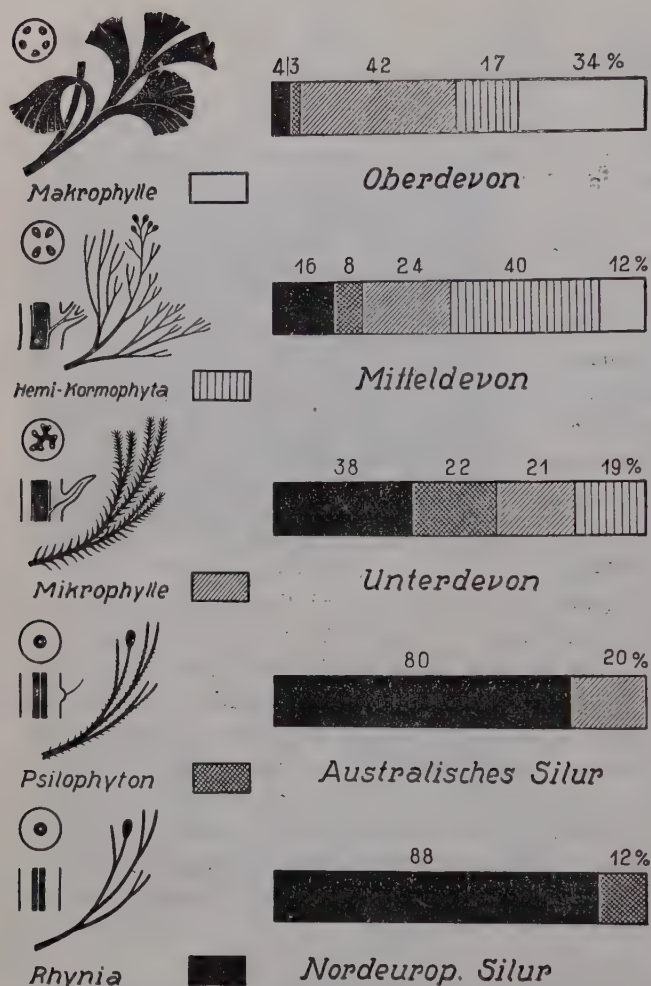
Fig. 3. A statistical proof for primitiveness of archaesyntelomes.
On the left: 5 types of oldest land-plants, on the right: their distribution (% of species) in the geological time. (See p. 403).

The cylinder of strand-initials is to be derived phylogenetically from the protostele of the archae-syntelomes by two elementary processes, viz. syngenesis and overtopping:

1) Parenchymatic *syngenesis* of meristeles (Fig. 5 C). This process may also be called *"basipetal shifting"* of stelar bifurcation. Its beginning is already visible for instance in *Taeniocrada Langi* from the inferior Devonian period (Fig. 6 A, found by F. Stockmans). The dichotomy of the stele seems basipetally shifted, when compared with the outlines of the telomes, and thence transverse sections show not a single stele but two steles, or two meristeles, as they are called in general (Fig. 6 B). By repeated basipetal shifting of the stele bifurcation a mesome may show 4 meristeles (Fig. 6 C); this structure is widespread in *Primofilices*. If only one of the two meristeles branches off, the threefold stele, a typical feature of early *Sphenophyta*, results.

We should keep in mind that, from a hologenetic aspect, the first change in this process is marked by the actual shifting of the determinating factors near the growing point.

2) Differentiation between cauline and foliar bundles by *overtopping* (Fig. 5B). This process corresponds entirely to the external overtopping of the shoot axis over the leaves and is its anatomical consequence. It is not possible to enter into particulars.

IV. The formation of metaxylem and other permanent tissue

We mentioned already that the formation of metaxylem, as a rule, takes place

in connection with the protoxylem and apparently under the determining influence of the latter.[3] This determining effect is manifested by the generally close relation of tracheidal elements in the metaxylem to the protoxylem, and the same usually applies to the secondary xylem. The different structure of the different steles results from a varying direction in the differentiation of the metaxylem which is demonstrated by some examples in Fig. 7.

In all cases the initial structure of the stele is the same, i.e. the cylinder of strand-initials ("Initialen-Bündelrohr"), which is represented by the strands of procambium or protoxylem (see Fig. 9 B). After this formation of the cylinder of strand-initials we see in Fig. 7 the following extensions of metaxylem:

Fig. 4.   Survey of stele types.
Light stippling: phloem; darker portions: metaxylem; black points: protoxylem.
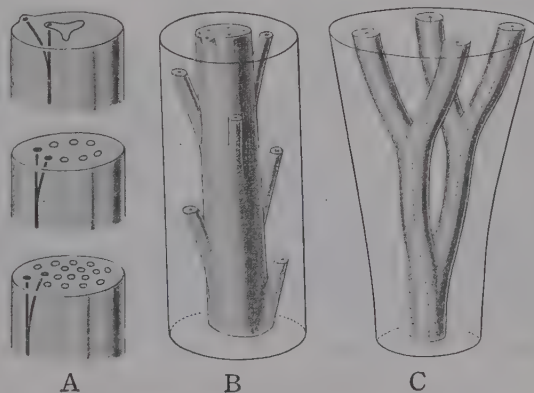
Fig. 5. A) Scheme of the cylinder of "strand-initials."
B) Overtopping and C) syngenesis of the stele.

in the *protostele* and *polystele* a *radial* extension of metaxylem (in the protostele the extension of metaxylem begins with one and in the polystele with several strand-initials);

in the *eustele* a *centrifugal* extension of metaxylem;

in the *actinostele* a *centripetal* extension of metaxylem;

in the *siphonostele* a *tangential* extension of metaxylem. Apparently there are determining factors which direct the growth of metaxylem in these directions.

3) For our question it is without relevance, if this determining impulse proceeds from the promtoxylem itself or, what is more probable, from its embryonal pre-stages.

V. Some characteristic instances

Asteroxylon Mackiei, M. Devon. (Fig 8).

The adult shoot has an actinostele with " mesarch " primary xylem in the " branches ".
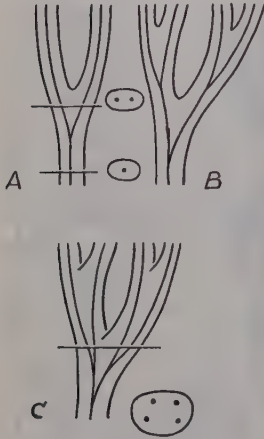
Fig. 6. A—C.   Fig. 6. D.

Beginning of syngenesis in *Taeniocrada Langi*. (See p. 404).

Protostele

Polystele   Eustele

Actinostele   Siphonostele   *Pietzschia*

Fig. 7. Directions of metaxylem extension. (See p. 405).

This implies an initially more or less centrifugal differentiation which is soon followed by a strictly centripetal extension; hence the xylem " branches " close up in the center of the shoot axis.

From the " branches " the leaf traces are forked off centrifugally. They are constructed in principle on the same model as the earlier initial strands of the axis (mesarch protostele as in Fig. 8B).

Three ontogenetical stages can be observed immediately:

a) The procambial stage in the bud of *Asteroxylon* (Fig. 8A). Here we have a characteristic procambial cylinder of initial strands, whose general structure corresponds to Fig. 1, middle. The cylinder of initial strands consists in shoot-buds with 4 to 6 cauline strands, well recognizable as dark, stretched cells filled with plasma. By one or two dichotomous furcations they detach procambial forerunners of leaf traces.

b) The stage of the first metaxylem is represented in principle by Fig. 1, upper left. As in the protostele, the metaxylem is formed radially around the protoxylem, i.e. in " mesarch " position to the latter.

c) This protostelic differentiation is stopped soon. In the cauline bundles the metaxylems (wide tracheids) grow only centripetally, conforming with the actinostele; accordingly the " branches " join in the center. The leaf traces remain in the earlier stage b.

The steles of the later *Lycophyta*, differ from *Asteroxylon* by an " exarch " posi-

tion of the prot o-
xylem. That means,
the determining
effect of the proto-
xylem acts only in
centripetal or tan-
gential direction, re-
spectively. In some
Sigillaria (for in-
stance *Sigillaria ele-
gans* Sternberg) the
centripetal determi-
nation of the meta-
xylem does not reach
the center of the
axis; hence we find
a "pith". This type
of the stele is called
"siphonostele", par-
ticularly since the
metaxylem forma-
tion goes, to a large
extent, also in tan-
gential direction.
Generally speaking,
the wood creates the
impression of a
cylinder.

*Lyginopteris old-
hamia* (Fig. 9).

In this upper
Devonian Pterido-
phyta equally three
ontogenetical stages
are to be discerned:

Fig. 8. A—B. *Asteroxylon*. (See p. 406).

a) Like in *Asteroxylon*, the procambial stage shows, in principle a distinct
cylinder of strand-initials. By tangential furcation the five cauline bundles detach
the foliar bundles, similar to the 2/5 position of the leaves. The latter divides fur-
ther in a characteristic way, namely in the same internode in which the cauline
bundles go on to bifurcate. Thence the whole stele reveals evidently the original
dichotomous organization of the protostele in *Psilophyta* (See Fig. 9 B).

b) The metaxlyem stage shows a radial structure and corresponds so far still
to the *Psilophyta*: we can speak of "mesarch" meristeles or bundles, respectively.
True, the metaxylem differentiating effect of the protoxylem goes somewhat more in
centripetal than in centrifugal direction.

Fig. 8. C.　*Asteroxylon*, part of Fig. 8. B.　(See p. 406).　　Fig. 8. D.　*Asteroxylon*, wood.
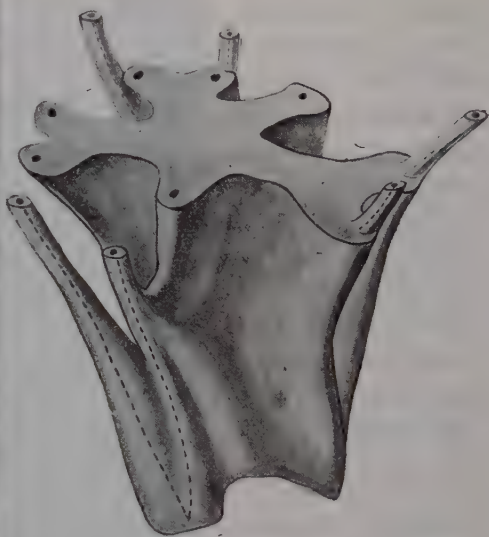
c)　In the stage of later wood formation, mainly that of secondary wood, a nearly exclusive centrifugal differentiation takes place.　Though, besides, we know some forms of *Lyginopteris* which, at least to some extent, have a radial differentiation within the secondary wood (similar to *Medullosa*).
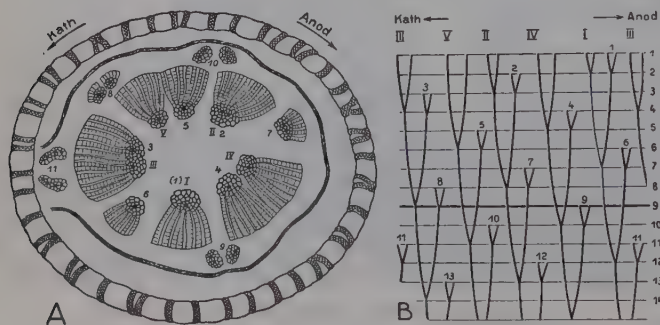
Fig. 9.　*Lyginopteris*. (See p. 407).

Other Pteridophyta. A centripetal differentiation of the metaxylem is rather conspicuous in some early *Pterophyta*. The typical actinostele[4] occurs in some *Primofilices* (for instance *Stauropteris*) though, in general, the axes of *Pterophyta* are characterized by polysteles, eusteles and siphonosteles; that means, the metaxylem is differentiated in different directions as described above (Fig. 7).

Medullation

In the earlier stelar theory (Fig. 10 A and B) a conception developed deviating from the notion demonstrated above.　According to that theory the medullation—

4) Thence the actinostele is the dominating type of the archae-stele; it is present in ancient *Sphenophyta* like *Sphenophyllum*, and we ascertained previously a fairly conforming type for *Lyginopteris*.

that is the formation of parenchymatous tissue within the protostelic center of the axis — is assumed to take place by "transformation" or "sinking in" of parenchyma into the axial center. These conceptions do not correspond to fossil findings nor to the ontogenetical processes of the ancestors. The (firstly proto-stelic) strands are always formed isolated, though they may merge in an actinostele by profuse development of centripetal metaxylem; this is the case mainly in archaic axes. The siphonostele, too,

A.   *"Sinking in" of parenchyma (JEFFREY)*

B.   *"Transformation" to medulla [KIDSTON & GWYNNEVAUGHAN]*

C.   *"Basipetal shifting" of stele-ramification [ZIMMERMANN]*

Fig. 10. Theories of medullation.

arises from subsequent fusion of tangential metaxylem within a cylinder of initial strands. Within the *Pterophyta* the Permian *Osmundales* seems to be the first to present the siphonostele. (They show a typical cylinder of initial strands that corresponds to the position of the protoxylem strands). Recent *Osmundales* possess isolated strands. That means, the determining power of protoxylem here is limited to isolated strands.

Zusammenfassung

1. Die Ahnengestalt der Stele, wie sie bei den ältesten Landpflanzen (zum Beispiel *Rhyniaceae*) ausgebildet ist, war eine Protostele mit centralem Protoxylem.

2. Bei ihrer phylogenetischen Abwandlung bildet sich in der Ontogenie in allen Stelen zunächst das "Initialen-Bündelrohr", ein Zylinder aus "kaulinaren" Prokambium— beziehungsweise Protoxylem — Strängen, von denen ähnlich gebaute Blattspuren ausgehen.

3. Die verschiedenen Typen der Stelen ergeben sich dann durch wechselnde Differenzierung der Folgegewebe (Metaxylem, Sekundärholz und so fort). Diese Folgegewebe werden offensichtlich ausstrahlend vom Protoxylem aus determiniert. Das verschiedenartige Aussehen der Stelen ergibt sich dann vor allem durch die wechselnde Richtung, in der die determinierende Wirkung sich zeigt.

For detailed references (also for Fig. 1—9) see

Zimmermann, W. 1954. Ueber die mikrophyllen "Psilophyten", ihre Entstehung und Bedeutung für die Stammesgeschichte. Paläont. Z. **28**: 56.

〃   1954. Das Homologieproblem, erläutert am Beispiel der Stelär-theorie. Ber. d. Dtsch. Bot. Ges., **67**: 312

〃   1955. Wie die Pflanzen an Land gegangen sind. Kosmos, **10**: 461.

〃   Phylogenie der Pflanzen. Jena 1930, 2. Auflage. In press.

The Morphology and Embryology of
Floerkea proserpinacoides Willd. with a Discussion on the Systematic Position of the Family Limnanthaceae

by P. Maheshwari and B. M. Johri[*]

The family Limnanthaceae comprises two genera, *Floerkea* and *Limnanthes*. The embryology of *Limnanthes douglasii* has been studied by Stenar (1925), Eysel (1937), Fagerlind (1939) and Mason (1951) but without any agreement on several critical points. More recently Mathur (1956) has investigated *L. douglasii* and *L. striata* and concluded that the embryo sac is tetrasporic corresponding to the pseudomonosporic, biphasic type[1] of Harling (1950). The earlier interpretation of Stenar and Eysel of an Adoxa type of development is not confirmed (*see* also Fagerlind, 1939).

Mason (1952) has recently conducted a systematic study of the genus *Limnanthes* and "utilized the traditional morphological approach, as well as chromosome studies of hybridization as embodied in more recent taxonomic techniques."

On *Floerkea*, a monotypic genus of North America, there is a general article by Russell (1919) and a short note by Johri & Maheshwari (1951) on its embryo sac and endosperm.

The systematic position of the family Limnanthaceae has been a debated question. Brown (1933) recognized some common features with the hypogynous families but did not assign a definite place to it. Engler & Prantl (1897) placed it in the order Sapindales and Hutchinson (1926) in the Geraniales. As will be shown here, none of these assignments is satisfactory and porbably the family needs to be raised to an ordinal rank.

Materials and methods

While on tour in the U.S.A. in 1946, one of us (P.M.) fixed some material of the plant from Madison, Wisconsin. Additional material was obtained in 1952 from Dr. L. Farquharson (Bloomington, Indiana) and Dr. L. A. Kenoyer (West Michigan College), and in 1952 from Dr. M. Fulford (Cincinnati, Ohio) and Dr. C. T. Mason Jr. (Stanley, Wisconsin). To all these persons we offer our most grateful thanks.

Buds, flowers and fruits were prepared and imbedded in the usual way. Sections were cut at 6-15 microns and stained in iron-haematoxylin as well as safranin and

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[*] Department of Botany, University of Delhi, Delhi 8, India.

[1] When only one of the four megaspore nuclei contributes to the formation of the embryo sac, the development is *pseudomonosporic*. The term *biphasic* means that two post-meiotic divisions occur before the organization of the embryo sac.

fast green. Both combinations gave good results. Dissections were also made of the endosperm at the globular stage of proembryo and stained with cotton blue in lactophenol.

Morphology

Floerkea proserpinacoides, popularly known as the 'false mermaid weed', is a short-sized (about 10 cm. high) annual, marshy herb with pinnately dissected, simple, alternate leaves.
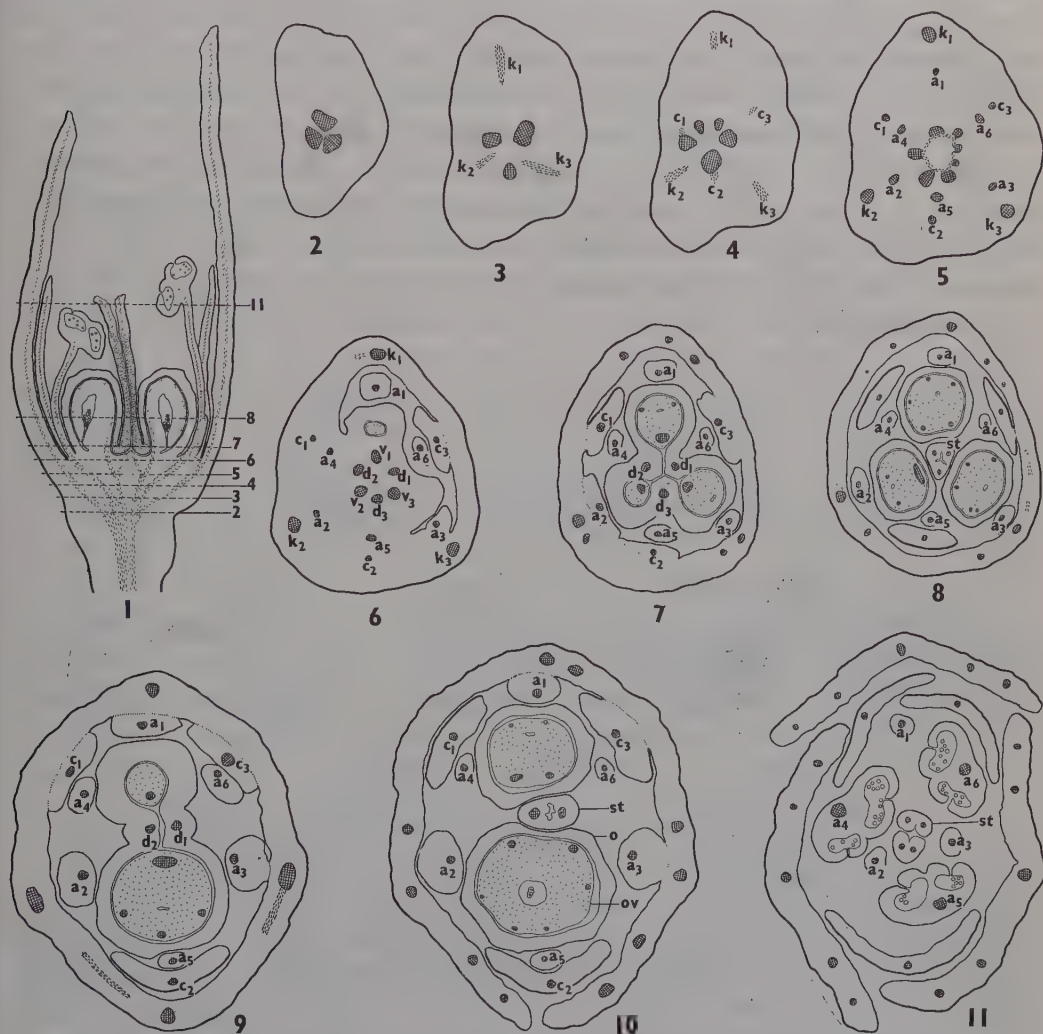
Figs. 1–11. Floral morphology and anatomy. (*a*, androecium; *c*, corolla; *d*, stylar bundles; *k*, calyx; *o*, ovary wall; *ov*, ovule; *st*, style; *v*, ovular bundles). Fig. 1. L.s. flower at mature embryo sac stage; the swollen portion of the filament on the right represents the basal gland. ×25. Figs. 2–8. Transection of a flower with tricarpellary gynoecium showing origin of vascular traces to various organs; approximately at level marked in Fig. 1, but of a younger flower. ×39. Figs. 9–11. T. s. older flower (bicarpellary gynoecium), more or less at levels, 7, 8 and 11 indicated in Fig. 1. ×39.

The solitary axillary flowers possess three large, imbricate sepals and three contorted petals (Figs. 1, 8, 10, 11); rarely there may be four sepals. The six stamens are arranged in two whorls, the outer alternating with the petals (Figs. 7-10) and having conspicuous basal glands. The gynoecium is usually tricarpellary but sometimes bicarpellary, with a gynobasic style (Figs. 1, 8, 10). The ovaries alternate with the petals, and each has a single basal-parietal ovule (Figs. 1, 8, 10).

The vascular supply of the flower is shown in Figs. 1-11. There are three bundles at the upper end of the pedicel (Fig. 2), alternating with which arise the three traces (k_1, k_2, k_3) to the sepals (Fig. 3). The traces to the petals (c_1, c_2, c_3) alternate with those of the sepals (Fig. 4). Further up each sepal shows one median and two lateral bundles, while the petal traces proceed undivided (Fig. 11). Next are the six staminal traces (a_1-a_6) which arise at about the same level (Fig. 5).

The remaining bundles of the central ring supply the gynoecium. One bundle enters each ovule, curves round and branches, the ramifications continuing up to the integument (Figs. 1, 7-10). Bundles alternating with the ovular supply enter the style (Figs. 6-8, 9, 10) and bifurcate in the region of the stigma (Fig. 11). As in *Limnanthes* (Saunders, 1928), the ovary wall is devoid of any vascular supply.
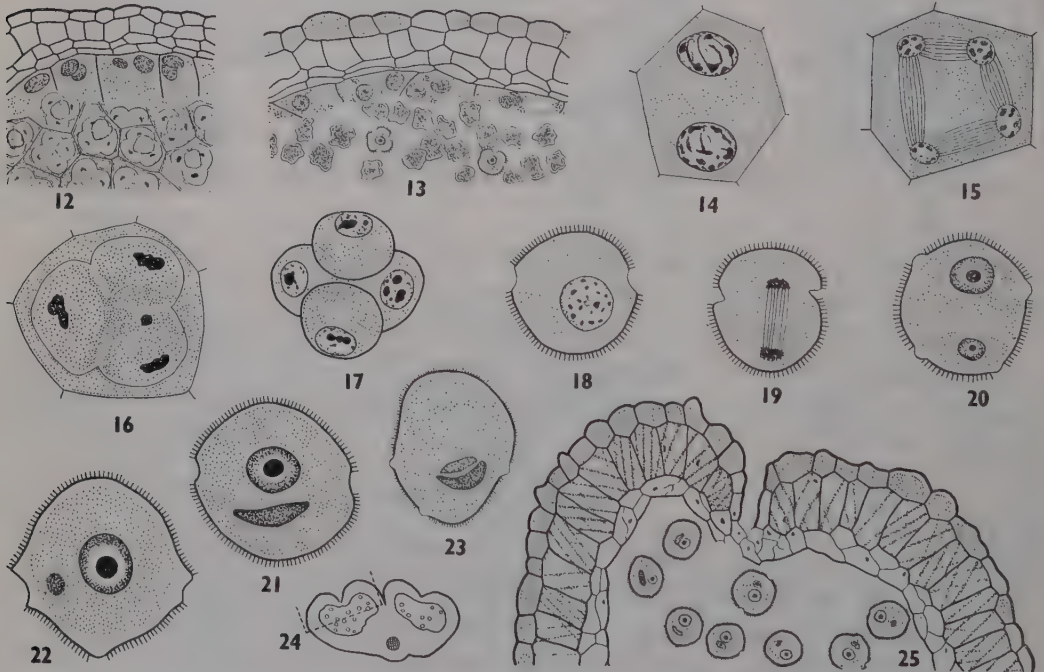
Figs. 12-25. Microsporogeneis and male gametophyte. Figs. 12, 13. Portions of anther lobes at tetrad and uninucleate pollen grain stage respectively. ×434. Figs. 14, 15. Microspore mother cells, Meiosis I and II. ×1585. Fig. 16. Cytokinesis by furrowing. ×1585. Fig. 17. Decussate tetrad. ×1585. Fig. 18. Uninucleate pollen grain. ×1585. Fig. 19. Division of microspore nucleus. ×1585. Figs. 20-23. Two-celled pollen grains. ×1585. Fig. 24. Outline diagram for Fig. 25. ×50. Fig. 25. Portion of anther lobe marked in Fig. 24, to show region of dehiscence. ×434.

Microsporogenesis and male gametophyte

Each microsporangium of the dithecous anther shows a group of hypodermal archesporial cells. An endothecium, two persistent middle layers and the glandular tapetum are derived from the primary parietal layer (Figs. 12, 13, 25). During reduction divisions the tapetal cells enlarge and become binucleate, but later the nuclei fuse (Figs. 12, 13). The inner tangential walls of the tapetal cells break down at the microspore stage (Fig. 13), and as the pollen grains mature the tapetum disorganizes.

The reduction divisions are simultaneous, secondary spindles are laid down during Meiosis II, and cytokinesis occurs by furrowing. Centripetal wedges formed by the special mucilaginous wall bring about quadripartition (Figs. 12, 14–16). The microspores are usually arranged tetrahedrally or in a decussate fashion (Figs. 16, 17); isobilateral tetrads also occur sometimes.

The microspores enlarge and the wall differentiates into an exine and an intine (Fig. 18). In some of the microsporangia there is widespread degeneration of the microspores (Fig. 13). On division the nucleus gives rise to a small generative and a large vegetative nucleus separated by a membrane (Figs. 19, 20). As the latter soon dissolves, the generative cell moves into the cytoplasm of the vegetative cell where it acquires a lenticular shape (Figs. 21, 22). In some pollen grains the vegetative nucleus had flattened and came to lie adjacent to the generative cell (Fig. 23). The mature pollen grains are rounded and 4-colporate, and the exine has warty projections (Figs. 20–23).

During maturation of the anther, the partition walls between the adjacent microsporangia break down (Fig. 24), and dehiscence occurs by longitudinal slits along the junction of the pollen sacs. In this region the epidermal and endothecial cells are smaller and thin-walled. Further, the former lack tannin and the latter are devoid of fibrous thickenings (Fig. 25). The stamens of the outer whorl dehisce earlier than those of the inner.

Ovule

The ovule is unitegmic and tenuinucellate, and the integument makes its appearance almost simultaneously with the differentiation of the archesporium. The curvature of the ovule is very rapid so that by the time the first meiotic division is over, it becomes anatropous (Figs. 26, 27). Besides the epidermis, the nucellus comprises only one more layer of cells on the sides (Fig. 30). This layer is crushed during megasporogenesis and the epidermis meets the same fate soon after.

The integument is massive and shows a conspicuous vascular supply (Figs. 1, 8, 10, 28). During its expansion the embryo sac consumes the adjacent cells of the integument (Figs. 42, 43).

Mathur (1956) reports that in *Limnanthes douglasii* a few cells of the integu-
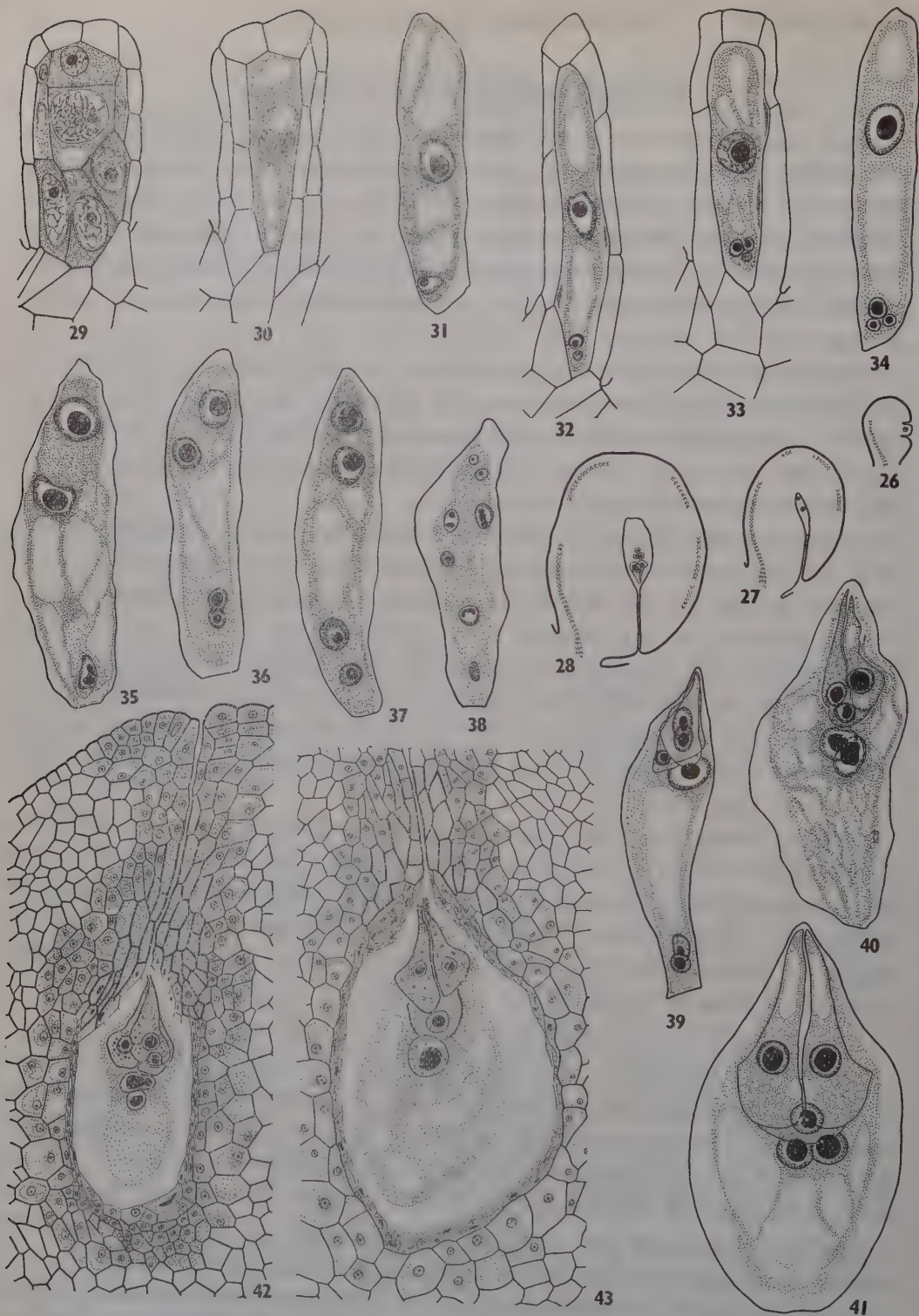
Figs. 26-43. Megasporogenesis and female gametophyte. Figs. 26-28. L. s. ovules at arche-sporial, 2-nucleate and mature embryo sac stage; Figs. 27 and 28 are outline diagrams for Figs. 31 and 42 respectively. ×49. Fig. 29. Multicelled archesporium. ×801. Fig. 30. Megaspore mother cell. ×801. Figs. 31-37. Stages in developmet of embryo sac; explana-tion in text. ×801. Figs. 38-39. Abnormal gametophytes; Fig. 38. ×801; Fig.39. ×528. Figs. 40, 41. Mature embryo sacs. ×528. Figs. 42, 43. Same, l. s. portions of ovules; note the thickened integumentary cells at the micropylar end. ×316.

ment at either end of the embryo sac become thick-welled. They have dense cytoplasm and appear to have a nutritive function. In *Floerkea proserpinacoides* such thick-walled cells are distinguishable only at the micropylar end (Figs. 42, 43).

Megasporogenesis and female gametophyte

There is a group of hypodermal archesporial cells (Fig. 29) but only one of them functions. As it differentiates into the megaspore mother cell, prominent vacuoles appear at either pole (Fig. 30).

The first meiotic division results in a markedly smaller nucleus which migrates to the chalazal end, and a larger one which stays on in the centre of the cell (Fig. 31). The smaller chalazal nucleus usually degenerates soon after formation (Figs. 32, 33). The upper nucleus undergoes the second reduction division and again produces one small nucleus which migrates to the chalazal end, and a larger nucleus occupying a central position (Fig. 32). The second nucleus at the chalazal end often divides to form two daughter nuclei (Fig. 33). Sometimes it is the first-formed chalazal nucleus which divides (Fig. 34) but such a behaviour is rare.

The upper larger nucleus has divided in Figs. 35-37. The two lower nuclei in Figs. 36 and 37 may have been formed as a result of division of the first-formed chalazal nucleus. It is also possible that they correspond to the two chalazal nuclei in Fig. 32; or, perhaps the first chalazal nucleus had already degenerated and disappeared and they are products of division of the second chalazal nucleus. In Fig. 35 these two nuclei seem to have fused. Unfortunately our preparations did not show any mitoses and therefore no definite conclusions can be made about the origin of the chalazal nuclei in Figs. 32-34, 36, 37 and 39.

The abnormal gametophyte represented in Fig. 38 is even more difficult to interpret. Since the nuclei vary considerably in size, it is probable that in this case there were irregularities in the meiotic divisions.

The two nuclei at the micropylar end undergo one more division and the daughter nuclei organize into the egg apparatus and the upper polar nucleus (Figs. 40-42). A lower polar nucleus is always present (Figs. 40-42); in origin it may either be the second chalazal nucleus (formed after Meiosis II of the upper larger nucleus) or its daughter nucleus. In Fig. 39 the large nucleus below the egg apparatus is probably the fusion nucleus and the two nuclei at the base may represent the two lower nuclei shown in Fig. 34 which have persisted for quite a long time. In Fig. 42 the nucleolus of the upper polar nucleus appears to be in a process of budding.

The mature embryo sac is conspicuously broad in the middle and narrow at the poles (Figs. 28, 40-43). The synergids are beaked and hooked and lack a basal vacuole (Fig. 41); the nucleus usually lies in the centre of the cell although sometimes it may be basally situated (*see* left synergid in Fig. 40).

Since walls are not laid down after the first or the second meiotic division, the development of the embryo sac is tetrasporic as also in the allied genus *Limnanthes*
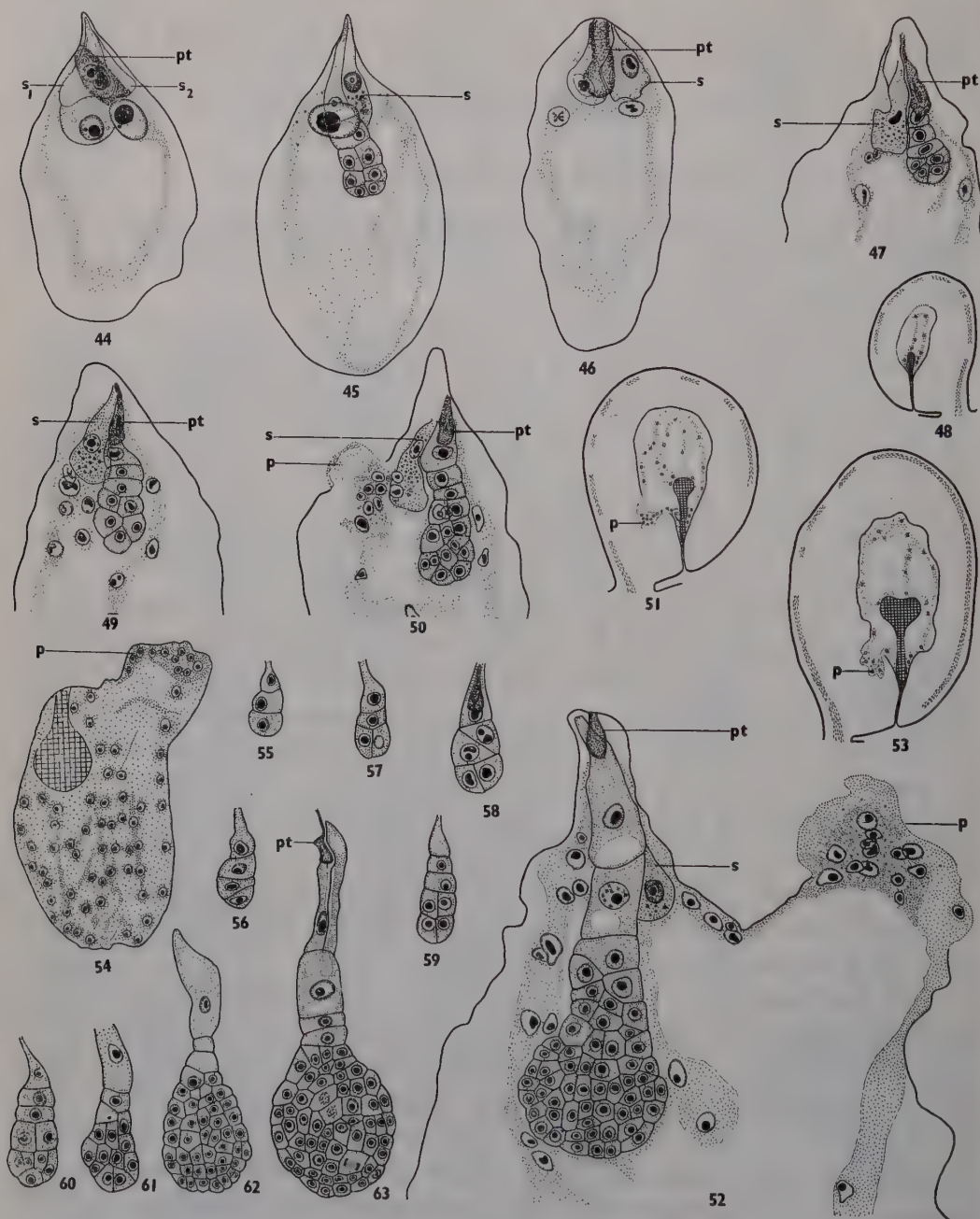
Figs. 44–63. Endosperm and embryo. (*p*, endosperm pouch; *pt*, pollen tube; *s*, persistent synergid). Fig. 44. Double fertilization. ×259. Fig. 45. Embryo sac with proembryo and undivided primary endosperm nucleus. ×259. Fig. 46. Same, showing zygote and two endosperm nuclei. ×259. Fig. 47. Upper part of embryo sac with persistent synergid, proembryo and free endosperm nuclei. ×259. Figs. 48, 51, 53. L.s. ovules at early and late globular, and heart-shaped stage of embryo; Figs. 48 and 51 are outline diagrams for Figs. 49 and 52 respectively. ×38. Figs. 49, 50, 52. Upper portions of embryo sacs; note the gradual aggregation of endosperm nuclei adjacent to the persistent synergid and formation of the pouch. ×259. Fig. 54. Endosperm and globular proembryo from whole mount. ×65. Figs. 55–63. Stages in development of the globular proembryo. ×259.

(Mathur, 1956). The nearest approach is to the Drusa type except that the activity of the nuclei situated at the chalazal end is very much restricted.

Neither in Mathur's material of *Limnanthes* nor in our preparations of *Floerkea* could there be seen any protrusion of the micropylar end of the 4–nucleate embryo sac as figured by Fagerlind (1939). In *Floerkea* the daughter nucleus resulting from the second meiotic division of the upper nucleus invariably migrates to the chalazal end (*see* Figs. 32–34) while in *Limnanthes* it is situated at a somewhat higher level. Antipodal cells were not observed and only in a few embryo sacs one or two nuclei were present in the chalazal end of the organized gametophyte (e.g. Fig. 39).

Fertilization

Double fertilization occurs as usual (Fig. 44) and remnants of the pollen tube persist until a late stage (Figs. 46, 47, 49, 50, 52, 63). Normally one of the synergids is destroyed during fertilization but sometimes both may remain intact (Fig. 44). The surviving synergid enlarges, takes a dense stain, and persists until the formation of the globular proembryo. Probably it has a haustorial function (Figs. 46, 47, 49, 50, 52). Fagerlind (1939) and Mathur (1956) also report a similar behaviour of one of the synergids in *Limnanthes*.

Endosperm

As a rule the primary endosperm nucleus divides earlier than the zygote. In Fig. 46 the first division has already taken place but the zygote is undivided. Fig. 45 shows the reverse condition where we have a well advanced proembryo but an undivided primary endosperm nucleus. Repeated nuclear divisions, which are not always synchronous, result in a large number of nuclei about 215 at early globular, 370 at late globular and 780 at the heart-shaped stage of the embryo. These nuclei take up a peripheral position (Figs. 48, 51, 53) although from the very beginning there is a tendency for an aggregation of the endosperm nuclei in the vicinity of the persistent synergid (Figs. 49, 50). The nuclei are imbedded in dense cytoplasm and the adjoining integumentary cells soon show signs of being corroded. Gradually a pouch is formed in this region containing 30–35 nuclei (Figs. 50–54). The pouch is always on the funicular side (Figs. 51, 53) and functions as an efficient absorbing organ. It has not been observed in *Limnanthes* by any of the investigators on this genus.

The peripheral layer of cytoplasm containing the endosperm nuclei invades the integumentary tissue all around the embryo sac so that the pouch is no longer distinguishable in older stages (compare Figs. 53, 75, 76).

The endosperm remains nuclear throughout and is consumed during the maturation of the embryo. No more than a darkly staining streak of its remnants persists between and around the cotyledons.

Embryo

The development of the embryo is shown in Figs. 55–63, a few stages being shown incidentally in Figs. 45, 47, 49, 50 and 52. The suspensor usually consists of a uniseriate row of 3–4 cells, but sometimes 6–9 cells may be formed. The uppermost cell elongates considerably, becomes vacuolated and may simulate a synergid (Figs. 52, 62). In one case it had undergone a longitudinal division (Fig. 63).

The progress of the embryo from the late heart-shaped to the mature stage is indicated in Figs. 75, 76, 77 and 64.
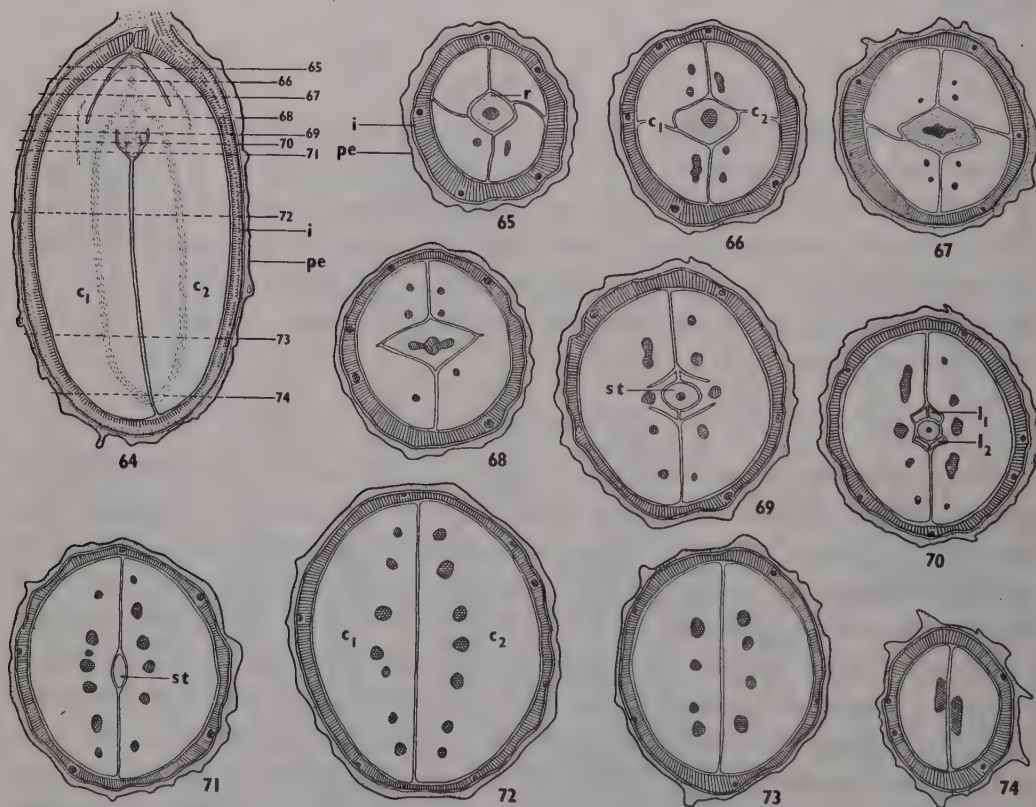
Figs. 64-74. Fruit. (c, cotyledon; i, integument; l, leaf; pe, pericarp; r, radicle; st, stem tip). Fig. 64. L.s. fruit. ×14. Figs. 65-74. T.s. fruit approximately at levels indicated in Fig. 64. ×14.

Seed and fruit

The fruit is a single-seeded, indehiscent achene (nutlet). The embryo occupies the entire seed cavity and comprises two large cotyledons, a small radicle, and the stem tip with two pairs of leaves (Figs. 64, 70). The reserve food material appears to be proteinaceous. A feature of special interest concerning the cotyledons is their backward extension and bifurcation on either side of the radicle so that in a

cross section at the upper end there appear to be four lobes (Figs. 65–67). The embryo has a well developed vascular supply which extends into the bifurcated portions of the cotyledons (Figs. 65–74). Neither Fagerlind (1939) nor Mathur (1956) examined the mature seeds of *Limnanthes* and it would be interesting to know if the cotyledons in this plant are like those of *Floerkea*.
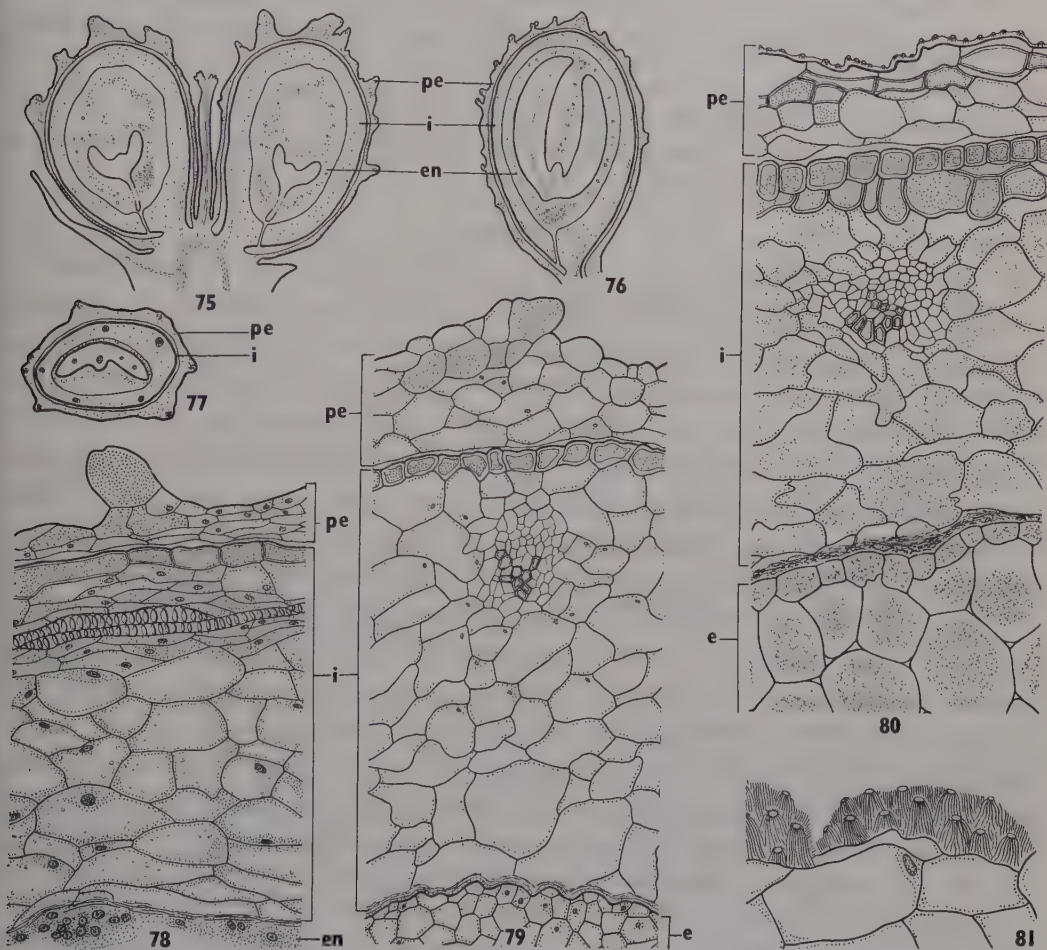
Figs. 75–81. Fruit, seed coat and pericarp. (*e*, embryo; *en*, endosperm; *i*, integument; *pe*, pericarp). Fig. 75. L.s. fruits, note the persistent gynobasic style. ×18. Fig. 76. Older fruit. ×18. Fig. 77. T.s. fruit.×18. Fig. 78. Portion of ovary wall, integument and endosperm enlarged from seed approximately of the same age as represented in Fig. 76. ×308. Fig. 79. Same, enlarged from Fig. 77; peripheral cells of the embryo are also shown, ×308. Fig. 80. Pericarp, seed coat, degenerated remains of endosperm, and portion of a cotyledon; enlarged from Fig. 66. ×308. Fig. 81. Thickenings on the outer tangential walls of epidermal cells of pericarp. ×515.

At the time of fertilization the integument consists of 14–16 layers of parenchymatous cells. The inner 6–8 layers are absorbed by the endosperm (Fig. 78), but the rest of the cells enlarge except in the neighbourhood of the vascular bundles

(Figs. 78, 80). In later stages all the cells show deposits of starch excepting the epidermis and hypodermis which become slightly thick-walled (Figs. 78, 80).

The ovary wall is 5 to 6-layered and localized growth at several points gives an uneven appearance to the outer surface (Figs. 75, 76, 78, 79, 64-74). In addition to a general enlargement of the cells, the epidermis and hypodermis acquire thick walls (Fig. 80). As in *Limnanthes* (Mathur, 1956) the outer tangential wall of the epidermis develops numerous pyramidal thickenings (Fig. 81) which gives the pericarp a specific pattern.

The pericarp and the testa remain adherent to each other. A few cells of the pericarp and the hypodermis of the testa are filled with 'tannin' and impart a reddish-brown tint to the fruit.

Discussion

The family Limnanthaceae is based on the type genus *Limnanthes* described by Brown in 1833. The material was taken to England from California by Douglas after whom the species *L. douglasii* is named. Brown also included the genus *Floerkea* in the above family, but added: "Examination proved these two plants to be so nearly akin that they might perhaps be included in the same genus."[*] Baillon (1871, 1878) observed tetramerous flowers in both *Floerkea* which is normally trimerous and in *Limnanthes* which is pentamerous and on this basis united them under the common name *Floerkea*. Several workers followed Baillon, while others maintained that these plants belong to separate genera (*see* Mason, 1952).

The affinities of the family Limnanthaceae are also unsettled. Brown (1833) wrote: "The place of this family is not absolutely determined, but it is suggested that in two remarkable points of its structure, namely, the presence of glands subtending the alternate filaments, and the existence of a gynobase, it more nearly approaches to Hypogynous familes than Perigynous, with which it has hitherto been associated."[*] Bentham & Hooker (1862) recognized the individual status of the two genera but placed them in a separate tribe, the Limnantheae under the Geraniaceae.

On the basis of the position of the seed, Engler & Prantl (1897) retained the two genera in the family Limnanthaceae and assigned it to the order Sapindales. On the other hand Hutchinson (1926) transferred it to the Geraniales which in their turn are regarded as derived from the Caryophyllales. While admitting considerable gaps between the two groups, he draws attention to some affinity between them through the Limnanthaceae.

Whereas most systematists accept the assignment of the Limnanthaceae to the Geraniales, from the morphological and embryological standpoint there are marked dissimilarities with the members of the Sapindales as well as the Geraniales as will be borne out from the following table (for literature references see Anantaswamy Rau, 1940; D'Amato, 1939; Erdtman, 1952; Johri & Ahuja, 1956; Kühn, 1928; Lawrence, 1951; Mathur, 1956; Schnarf, 1931; and Venkateswarlu & Narayana, 1955):

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* Quoted from Mason (1952).

CHARACTER	LIMNANTHACEAE	SAPINDALES	GERANIALES
Flowers	Regular, perfect; petals contorted.	Often irregular; unisexual.	Often irregular; usually hermaphrodite, sometimes unisexual.
Androecium	Stamens in 2 whorls, outer alternates with petals and has basal glands.	One whorl, inserted upon a disc or between disc and ovary.	Typically two whorls, the outer sometimes missing; disc glands often present.
Gynoecium	Ovaries separate from one another; style gynobasic; only one basal-parietal, ascending ovule with micropyle pointing downwards.	Syncarpous; ovary entire or lobed; axile placentation; ovules pendulous, dorsal raphe and micropyle pointing upwards; or erect with ventral raphe and micropyle pointing downwards.	Syncarpous; ovary entire or lobed; axile placentation; ovules 1–2 in each locule, pendulous with ventral raphe and micropyle pointing upwards; or erect with dorsal raphe and micropyle pointing downwards.
Anther tapetum	Glandular.	Glandular; amoeboid in some plants.	Glandular; amoeboid in some plants.
Pollen	2–4 colporate.	Usually 3–colporate.	Usually 3–colporate.
Ovule	Anatropous; unitegmic; tenuinucellate; integumentary bundles present.	Anatropous; bitegmic; crassinucellate; tenuinucellate in Balsaminaceae; integumentary bundles present only in rare cases.	Anatropous; bitegmic; crassinucellate (Linaceae, Oxalidaceae); integumentary bundles present in Euphorbiaceae.
Embryo sac	Tetrasporic, but development different from that in tetrasporic members of Euphorbiaceae and Malpighiaceae.	Mostly monosporic; Allium type in *Hydrocera triflora* (Balsaminaceae).	Mostly monosporic; tetrasporic in Malpighiaceae; mono-, bi- or tetrasporic in Euphorbiaceae.
Endosperm	Nuclear; in *Floerkea* a coenocytic pouch is formed adjacent to the funicular side.	Nuclear; a large multinucleate micropylar haustorium is formed in *Hydrocera triflora*.	Nuclear; a chalazal endosperm haustorium occurs in *Aegle* (Rutaceae) and *Suriana* (Simarubaceae).
Embryo	Straight; cotyledons large; there is an upward extension and bifurcation of the cotyledons in *Floerkea*.	Curved; cotyledons crumpled; nucellar embryony occurs in Anacardiaceae.	Straight; nucellar embryony occurs in Rutaceae.
Seed	Exalbuminous.	Exalbuminous, but sometimes albuminous.	Exalbuminous, sometimes albuminous.
Fruit	Schizocarpic, 1-seeded achene (nutlet); indehiscent; style persists until a late stage.	Various types.	Capsular; dehiscent; style often persists in fruit.

Thus, the Limnanthaceae differ from both the Sapindales and the Geraniales in having (1) only herbaceous members, (2) gynobasic style, (3) unitegmic, tenuinucellate, basal-parietal ovules, and (4) an unusual type of tetrasporic embryo sac. The Sapindales are mostly shrubs and trees, the leaves are usually compound, the flowers are often unisexual, the stamens are in a single whorl, and the embryo is

curved with crumpled cotyledons. The Sapindales as well as the Geraniales lack a gynobasic style, the ovules are penduluos on axile placentae, the pollen grains are 3-colporate, ovules are bitegmic and crassinucellate, the development of the embryo sac is predominantly monosporic, and nucellar embryony occurs in some of the families under both the orders.

Concerning pollen morphology, Erdtman (1952) remarks: " The grains in Limnanthaceae are not similar to those in Balsaminaceae, Convolvulaceae, Coriariaceae, Geraniaceae, Hydrocaryaceae, Nymphaeaceae-Cabomboideae, Sapotaceae and Tropaeolaceae."

Judging from present evidence, then there are very few features common to the Limnanthaceae and members of the Sapindales and Geraniales. Since some of the families usually included in the above orders have already been given an ordinal rank (*see* Hutchinson, 1926; Lawrence, 1951; Core, 1955), we are of the opinion that the Limnanthaceae may also be given a similar status and placed in a new order Limnanthales.

Summary

Floerkea proserpinacoides is a monotypic North American plant belonging to the family Limnanthaceae. It bears solitary trimerous flowers. The stamens are arranged in two whorls of which the outer alternates with the petals and has basal glands. The gynoecium is usually tricarpellary, the ovaries are separate from each other and the style is gynobasic. There is a single, basal-parietal, ascending ovule in each ovary.

The anther wall comprises the epidermis, fibrous endothecium, two persistent middle layers and glandular tapetum. The reduction divisions are simultaneous, cytokinesis occurs by furrowing and the tetrads are decussate or tetrahedral. The pollen grains are tetracolporate and are shed at the 2-celled stage.

The ovule is anatropous, unitegmic and tenuinucellate with an integumentary vascular supply. A group of archesporial cells differentiates in the young nucellus but only one of them functions as the megaspore mother cell. A parietal cell is not cut off and the development of the embryo sac is tetrasporic. The mature gametophyte shows the egg apparatus, and two polar nuclei; rarely one or two antipodal nuclei are also seen.

The endosperm is nuclear and walls are not laid down. The endosperm develops a pouch-like haustorium at the micropylar end towards the funicular side.

The zygote divides transversely. The mature embryo has a short radicle. The cotyledons have backward extensions which become forked.

The testa consists of 8-10 and the pericarp of 3-4 layers. The pericarp has an uneven surface and the outer tangential wall of the epidermis develops pyramidal thickenings. The epidermis and hypodermis of testa and some cells of the pericarp contain 'tannin'. The fruit is an indehiscent achene (nutlet).

The morphological and embryological features of the Limnanthceae do not support its inclusion either in the order Sapindales or the Geraniales. It is suggested that this family may be given an ordinal rank under the name Limnanthales.

We are indebted to Mr. S. N. Dixit who prepared most of the illustrations, and also to Mr. S. P. Bhatnagar and Mr. Hardev Singh for other assistance in the preparation of this paper.

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* Originals not seen.

A Comparison of Two Mesozoic Fern Floras

by Tom M. HARRIS*

1. Introduction

It has been my good fortune to study two large and well preserved Mesozoic floras. One[1] is from Scoresby Sound in East Greenland at Latitude 70° N and is of Rhaetic and basal Liassic age. The other is from Yorkshire in N. E. England at Latitude 54° N and is of Lower Oolite age (Bathonian and Bajocian). Both floras are preserved in an area where a large delta was formed and able to keep pace with subsidence and marine transgression and the fossils are largely the plants growing in the swamps of the delta. In both, large Equisetales flourished in lagoons and are the commonest fossils. In both, Gymnosperms are the chief land plants but ferns are very common and may have been the main herbs of the forest.

The two floras have been collected and studied and in the same way, but collecting in Yorkshire has been far more extensive and many have contributed. The Greenland plant beds are, however, easier to work, and our knowledge of some of the inadequately known species can be supplemented by what we know of them in the Rhaeto-Liassic floras of S. Sweden and of S. W. Germany.

The two floras are thus as fully comparable as could well be with present knowledge and their comparison should illuminate plant development during the Liassic period, perhaps 10−20 million years.

The Greenland flora has been described by me in a series of papers between 1926 and 1935; the Yorkshire one[2] is presently to be redescribed in a new British Museum catalogue.

2. Identical species

It has long been known that certain Rhaetic and Oolitic ferns are very similar. Some authors have identified them, but others (including myself) have separated them, feeling that they would presently be demonstrated to be different. However, now I have studied both sets of specimens as fully as I could I can only say that no difference has emerged, but on the contrary I have found new and unsuspected points of agreement.

Whatever views others hold about the meaning of the 'species' in fossil plants, to me these indistinguishable specimens of different age are the same species.

Those species of ferns common to the Greenland Rhaeto-Liassic and the York-

1) Harris, T. M. 1937. The Fossil Flora of Scoresby Sound, East Greenland. Medd. om Grønland, Bd. 112. Nr. 2 (Earlier references are given).
2) Harris, T. M. in preparation. Catalogue of the Yorkshire Jurassic Flora.

shire Lower Oolites are :—

Todites williamsoni   (including *T. goeppertiana*)

Todites princeps   (including *T. leckenbyi*)

Phlebopteris polypodioides   (including *P. affiinis*)

There is also *Selenocarpus muensterianus*, unknown in Greenland but of the German Liasso-Rhaetic and now found in Yorkshire.

T. williamsoni may range even further for similar ferns are known from the Middle Trias and Upper Jurassic but they have not been studied microscopically. Although outside this comparison, I would mention that certain ferns of the Lower Oolite extend into the Lower Cretaceous, namely :—

Matonidium goepperti   *Phlebopteris woodwardi*

Phlebopteris dunkeri   *Hausmannia dichotoma*

The two last appear at some stage in the Lias of Bornholm.

These statements involve personal judgment about various unequally known specimens and some authors would give different ranges. They are merely my judgment; which I can summarise by saying that a good many fern species lasted for about half the Jurassic but few, if any, Gymnosperms lasted so long.

3. Comparison by families

A. Marattiaceae. This family seems to have declined catastrophically at about the end of the Trias; in the Rhaetic we still see a few *Asterotheca* species and *Danaeopsis*, but by the Lower Lias there is only *Marattiopsis* which persists into the Jurassic. There is one *Marattiopsis* species in Greenland and a very similar one in Yorkshire. Like many species of *Angiopteris* (but not *Marattia*) they show fibrous strands between the veins.

Nathorstia is usually included in the Marattiales and two Yorkshire ferns were once referred to it (by Hirmer and Hoerhammer) but I believe they are true species of *Phlebopteris* and belong to the Matoniaceae. Balsam transfers indicate that they have sori of separate sporangia and not synangia. The Cretaceous species of ' *Nathorstia* ' deserve re-examination by this method.

B. Osmundaceae. This family is heavily represented in Greenland by six species and five in Yorkshire. In each flora there is one, rare, *Osmunda*-like form and all the others have fertile pinnules like *Todea*. There are, however, differences for as Thomas first pointed out the sporangia in *T. williamsoni* (and indeed in all Jurassic species examined) have a larger and more apical group of thick walled cells. These Jurassic *Todites* species fall into three groups; one with more or less Neuropterid venation (including *T. williamsoni*, one with purely Pecopterid venation and rather specialised fertile pinnules ' *Cladotheca* ' and finally *T. princeps* with Sphenopterid pinnules showing anadromic branching an unusual feature in Jurassic or older ferns.

Cladophlebis (or *Todites*) *denticulatus* deserves special mention for its supposedly

wide distribution. It was described from really excellent Yorkshire specimens in 1828, and then as other Mesozoic floras came to light more or less similar fragments were referred to it. Later good *Cladophlebis* leaves were discovered in these floras and distinguished as new species, but the indeterminable fragments were left in *C. denticulatus* which has thus become a repository for rubbish; I suppose all early described fossils are in danger of this. I have no idea now many of the 50 or so localities from which *C. denticulatus* has been recorded really contain it, certainly none outside Yorkshire disclose the characteristic fertile leaf. I am sure that vigorous search for fertile leaves would help. They are seldom conspicuous fossils and the sporangia are nearly always concealed, waiting to be exposed as a balsam transfer.

C. **Matoniaceae.** This family maintained or even improved its position during Liassic time; in Greenland there are three species, (but some others elsewhere in Europe) and in Yorkshire there are six; though as mentioned earlier two of these have on occasion been included in *Nathorstia* and referred to the Marattiales. In this family also balsam transfers of fertile leaves give most interesting results. They prove that the placenta in some species of *Phlebopteris* is flat, in others it is more prominent that Hirmer and Hoerhammer had reason to suppose and in *P. woodwardi* its end is distinctly expanded (exactly as in their diagram of *Matonidium*, while in *Matonidium* itself it is much further expanded than they supposed and agrees with the living *Matonia pectinata*. The genera will presently need to be revised, and it is clear that if evolutionary enlargement of the placenta occurred in the family, it was completed rather early.

D. **Dipteridaceae.** This family has two very different sections. One the Camptopterideae declined drastically during the Lias for in Greenland it has six species some very common, but in Yorkshire there are but two, both infrequent. This decline seems general, for there are few if any species younger than Middle Jurassic. These two Oolitic species are very like Rhaeto-Liassic species, thus there is no evidence of evolution but only of extinction.

The other section called the Dipterideae has *Hausmannia* alone, a genus sparingly represented in both floras. *Hausmannia* sporangia demand further study now we have the transfer method. None of the fertile fragements I have seen retain sporangia, but specimens are known which certainly have them. The spores (at present undescribed) should be interesting, I believe *Dipteris* spores are wedge shaped and have a perispore; those of the Camptopterideae are triradiate and smooth.

E. **Schizaeaceae.** This small family is unrepresented in Greenland (but one or two species do occur in the German Rhaeto-Liassic), while in Yorkshire there is the well known *Klukia exilis* and also *Stachypteris spicans* which on reinvestigation appears to be close to *Lygodium* and has nothing to do with the Loxsomaceae. This family has most characteristic spores and I am told (by Mr. A. Couper) that isolated spores of unknown Schizaeaceae occur widely through the Jurassic.

F. **Gleicheniaceae.** This family may be represented in the Palaeozoic by *Oligo-*

carpia but in the Rhaetic and Jurassic if truly represented at all is very rare. There is one species known from small fragments (and therefore not very securely determinable) from Greenland, nothing whatever from Yorkshire. It suddenly becomes abundant in the Cretaceous, as though invading from some other region.

G. Dicksoniaceae. The rise of this family during the Liassic is spectacular. There is not one species in Greenland nor so far as I know in any Rhaeto-Liassic flora. In the Yorkshire inferior oolite there are ten species, some very abundant and some of these appeared rather earlier, during the Liassic of Bornholm. The Dicksoniaceae is thus an invading family and not a natural development of any earlier ferns we know of.

The modern Dicksoniaceae is divided into the Thyrsopteroideae and the Dicksonioideae and this I believe (with some reservations) to be true of the family in the Jurassic but the relative proportion of the two sections is reversed. The Thyrsopterideae (with a nearly round sorus and indusium) have eight species in Yorkshire but the Dicksonioideae (with a two-valved indusium) has only two species and both are rare. Both appear to be just like the modern genus *Dicksonia* (sensu lato) but there is not the information on which to compare it with the three subgenera now recognised.

I suspect that the very incompletely known ferns called *Gonatosorus* may belong to *Dicksonia*, for fossil *Dicksonia* sori look like the figures of *Gonatosorus* when imperfectly exposed. This could be cleared up by transfers.

The Thyrsopterideae are taxonomically difficult, and the preparation of spores and transfers does not fully penetrate the difficulty but rather takes one further into it. Two species stand apart, *Eboracia lobifolia* with a cylindrical indusium and *Coniopteris arguta* with a cup-shaped indusium and long placenta; it also seems worthy of generic rank. The remainder are at present included in *Coniopteris* (a genus of remarkably illegitimate origin even among fossil plants).

They form a series ranging from *C. murrayana* which Brongniart designated as Type to *C. hymenophylloides* at the other extreme. Brongniart described *Sphenopteris murrayana* in 1834 and included in it a fertile leaf with a superficial Aspidium-like sorus which I believe distinct, and which was presently lost to sight. In 1849 when he made *Coniopteris* he referred the fertile fragments *Tympanophora simplex* of Lindley and Hutton (1835) to it, again wrongly as I believe. It proves that *C. murrayana* is a fern with a large broadly triangular leaf. The fertile pinnules are only slightly reduced and bear small flattened sori. The indusium though flat is continuous and the minute placenta bears few sporangia, only two or three being ripe at the same time. At the other extreme *C. hymenophylloides* has narrow leaves, the fertile pinnules are strongly reduced and bear fairly large cup-shaped sori. The placenta bulges and bears about fifty sporangia which ripen together.

Other have treated these ferns very differently. Rather recently (1944) Kobayashi and Yoshida described a Manchurian leaf very like *Coniopteris burejensis* as

Odontosorites heereanus comparing it with living species of *Odontosoria*. The main difference between *Dicksonia* and *Odontosoria* (and several other genera of the Lindsayeae) is in the annulus which in *Lindsaya* is interrupted by the stalk but in *Dickonsia* continues past it. In the fossils this is most difficult to demonstrate even with transfers, because in the intact sorus each sporangium overlaps the base of the next and the lower ones are hidden by the indusium. Loose but associated sporangia may show a continuous annulus but their attribution is debatable. It is always thus in fossil botany, the point at which evidence fails is the one where its need seems greatest.

Others have placed *Coniopteris*-like ferns in *Davallia*, for instance Teixeira who described a lower Cretaceous species looking very like *C. hymenophylloides* as *Davallia alfeizeranensis*. This may be correct, but here proof should be much more easily obtained. The spores of *Davallia* are bilateral as in most other recent ferns but in *Dicksonia*, *Odontosoria* and *Coniopteris* and its allies they are very different, being three rayed.

This Mesozoic fern group deserves much wider attention. Transfers of sori and spore preparations may not give all we want, but they give much and their use in other floras may well throw light on the origin of many of our modern ferns.

H. Aspidioid ferns. Very few Jurassic ferns have sori at all like those of the Aspideae, and even Cyatheaceae (without the indusium) are very few till the Cretaceous. However, Brongniart (1828) figured one Yorkshire fragment where such a sorus is suggested, referring it though wrongly to *Sphenopteris murrayana*. The species has been found again, direct observation shows no more than Brongniart's figure but transfers reveal a round sorus of numerous, small sporangia covered by a slightly excentric indusium intermediate between that of a typical *Polystichum* and a typical *Nephrodium*. The sporangia contain 48 spores, again I cannot be certain whether the annulus continues past the stalk. One fact conflicts with classification among the aspidioid ferns, the spores are smooth and triradiate and not bean-shaped and rough as would be expected. The spores are more like those of *Coniopteris* and such modern genera as *Cyathea*, *Dicksonia* and the Lindsayoideae. Apart from this I would have referred this Yorkshire fern without reservation to the Aspideae and possibly have expressed views about the evolution of the indusium. In any case it is at present isolated, its position may be clear when transfers are made of fertile ferns of other floras and it begins to have allies.

I. Unassigned ferns. In all floras there are fern species which are only known from sterile material and therefore placed in form genera and only classifiable by a rash guess. In both the floras I am comparing there are three of these species, mostly Pecopterid (*Cladophlebis*) but in Yorkshire there is one Sphenopterid fern with a delicate lamina one cell thick as in *Hymenophyllum*.

In these floras the percentage of these disgraceful species is about ten per cent, the fact that it is so low is due largely to the transfer method which reveals

good sori on seemingly unpromising material. It is too much to hope that we shall ever eliminate all unassigned ferns for there are always the rare species known only from a single specimen which happens to be sterile; but I see no reason why the figure should ever be much higher than this ten per cent.

4. General conclusions

A. Number of species. A point which the Yorkshire ferns has forced on my attention is that the number of recognised species in the flora tends to diminish as work proceeds. In 1875 Phillips recognised some 45 species in the Yorkshire flora. In 1900 Seward reduced them to fifteen, rightly as I think in nearly all cases. The fifteen additional species represent new discoveries, the result of much hard work. Transfers and spore preparations are particularly useful here for while pinnules vary in different parts of a leaf, epidermal hairs and spore forms do not. It is quite otherwise with Gymnosperms, cuticle preparations reveal unsuspected differences. It follows that the number of species in floras invesnigated by different methods are hardly useful for comparison. The number of species in the Yorkshire and Greenland floras, recognised by me is, however, rather similar (30 Yorkshire, 23 Greenland). Doubtless this Greenland figure would be increased if the amount of rock examined had been as great as in Yorkshire.

B. Principal changes. While certain families (Osmundaceae and Matonicaceae) roughly maintained their position through the Liassic, others (Camptopterideae) lost heavily and are approaching extinction while one at least, the Dicksoniaceae surges in like an invader. We know nothing of the origin of this family, it first appears at approximately Middle Jurassic times in many parts of the world and very quickly reaches full development. In a very small way its rise recalls the rise of the Angiosperms in the Cretaceous. Indeed it seems true of many families or at least of the way they play their parts in public.

C. Evolution. The extinction of some species and their replacement by new ones has more the appearance of invaders surplanting an aboriginal population than gradual evolution. I have looked hard for evidence of gradual morphological change but have not found it. Among species which I regard as common to Greenland and Yorkshire, there is *Phlebopteris polypodioides* in which secondary vein meshes are perhaps a little commoner in Oolitic than in Rhaetic specimens, but there is certainly no great difference. In *Todites williamsoni* pinnules with wavy margins are certainly commoner in Yorkshire than in the Greenland Rhaetic. I suspect that this difference may be more like that separating geographical races of a species than a difference caused by time because the material from the Rhaetic of Tonkin is slightly different again, specimens with rounded fertile pinnules being remarkably prominent. These differences seem trifling; they look as though they might be shown to be statistically significant by suitable analysis but this does not make them worthy of specific rank. These differences have nothing to do with the major morphological changes which must have happened in the evolution of the modern fern genera.

Some Chemical Aspects on Anthocyanin Coloration Caused by C and Sp Allelomorphic Series of Genes

Genetical Studies on Rice Plant XX*

by Seijin NAGAO,** Man-emon TAKAHASHI** and Takao MIYAMOTO**

長尾正人**・高橋万右衞門**・宮本隆夫**: C 系及び Sp 系複対立遺伝子に関する
花青素着色の化学的性状 (稲の交雑に関する研究 XX*)

Introduction

In the past, it has been assumed that the expression of various hues and shades of anthocyanin coloration in rice plant necessitates the existence of a modifier which controls the color intensity in addition to the chromogen and its modifier, which in turn converts chromogenic substance to anthocyanin (Nagai 1921, Yamaguchi 1926, Chao 1928, Jones 1930 etc.).

However, the authors have not yet arrived at the occasion where any other intensifying modifier is required, so far as our cross experiments are concerned (Nagao and Takahashi 1947, 1951). A detailed account on this interpretation, being tedious, is abridged here, and is summarized as follows.

According to the authors' genic scheme, anthocyanin coloration depends on the complementary effect of gene C and Sp. The C is responsible for the formation of chromogen, and the gene Sp exerts its modifying effect on C and turns the chromogen to anthocyanin. Five alleles, $C^B > C^{Bp} > C^{Bt} > C^{Br} > C^+$, have been discovered at the C locus and three, $Sp > Sp^d > Sp^+$, at the Sp locus. Therefore, the expression of anthocyanin color character can be said to be determined by the combination of alleles of the C and Sp loci. The following is the relation of hue and shade arranged according to color intensity in the ascending order.

    violet................red................pink................colorless

$$C^B Sp > C^{Bp} Sp > C^B Sp^d > C^{Bp} Sp^d > C^{Bt} Sp > C^{Br} Sp > S^{Bt} Sp^d > C^{Br} Sp^d > Sp^+$$

Coloration occurs primarily in the glume apex, namely the apiculus, as a result of the interaction of the said two kinds of genes; and thereupon if the gene Rp, Pl or Pn, which are regarded as the distributing modifier for anthocyanin coloration, coexist with C and Sp; the glume, the leaf or the node are also colored with anthocyanin respectively.

To take an objective view of genic interpretation on these colorations, it is necessary to investigate not merely an account of the genic analysis of their characteristics by means of hybridization experiments, but also consideration of the biochemical analysis of the said characteristics. In the present paper the authors intend to deal with some chemical natures on anthocyanin coloration due to the presence of *C*, *Sp* and their modifiers *Rp*, *Pl* and *Pn*.

Before going further, the authors wish to express their gratitude to Prof.Y.Nakamura, who has tendered many suggestions from his speciality, and Dr. K. Hayashi for the use of an authentic specimen which was exceedingly valuable in identification of the anthocyanin concerned. Expense of this work was partly defrayed from a grant for Fundamental Scientific Research of Ministry of Education.

Materials

The details are given in the sections of experimental results, but in general the investigation was accomplished using strains which have been bred true for coloration with regard to the *C* and *Sp* series of multiple allelomorphic genes and their distributing modifiers, and in a few cases some genotypic individuals derived from one F_2 population were used.

Experimental Results

I. In order to determine whether various color intensities, *i. e.* blackish purple to rose pink, are caused by identical anthocyanins or not, several genotypes in connection with *C* and *Sp* alleles were examined by means of paper chromatographic method.

The glume apices and their awns were taken and collected two to three days after flowering, and the anthocyanins contained in above parts were extracted with 1 % solution of hydrochloric acid. The extracted solutions were spotted on filter papers (No. 50 of Toyo Roshi Co., Ltd.) and dried at 26° C. Separation of anthocyanins was conducted by the use of a mixture of *n*-butanol, glacial acetic acid and distilled water in a ratio of 4 : 1 : 2, as advocated by Bate-Smith (1948). After one dimensional separation of the extracts, the filter papers were dried at room temperature, and Rf values of anthocyanins were observed and calculated. The results are as shown in Table 1.

In this table it is pointed out that almost all genotypes show similar values of Rf, ranging from 0. 41 to 0. 43.

The same Rf values were obtained in the extracts from another colored parts in which the localization of color depended on the presence of distributing genes *Rp*, *Pl* and *Pn*, under the coexistence of fundamental apiculus genes *C* and *Sp*. This result is given in Table 2.

Table 1. Rf values of anthocyanins extracted from apiculus and awns of different genotypic individuals or strains (some combinations of alleles of the C and Sp loci).

Genotype	Hue and shade of color[1]	Strain or population no.	Part selected for examination	Rf value
$C^B\ Sp$	Blackish purple	A-2	apiculus and awns	0.42
		A-58	"	"
$C^{Bp}\ Sp$	Pansy purple	A-107	"	0.41
		A-77	apiculus	0.43
$C^B\ Sp^d$	Aster purple	A-18	"	0.42
$C^{Bp}\ Sp^d$	Amaranth purple	A-83	"	"
$C^{Br}\ Sp$	Rose red	A-5	apiculus and awns	"

1) Refer to Ridgway's Color Standards.

Table 2. Rf values of anthocyanins extracted from organs other than apiculus and awns.

Distributing modifier	Mode of color distribution by modifier	Basic genotype	Strain or population no.	Part selected for examination	Rf value
Rp	Entire glume and rachilla	$C^B\ Sp$	A-58	glume	0.42
		$C^{Bp}\ Sp$	A-107	"	0.43
Pl	Entire leaf blade and sheath, ligule, pulvinus, auricle, stem and node	$C^B\ Sp$	D-25	leaf blade	0.42
		$C^{Bp}\ Sp$	A-77	"	"
				leaf sheath	"
				node	0.43
			A-38	leaf blade	0.42
			N-45	"	"
		$C^B\ Sp^d$	N-4	"	"
Pn	Leaf margin, leaf sheath, pulvinus, auricle and node	$C^B\ Sp$	A-65	leaf margin	0.41
			A-2	"	0.42
				ligule	"
				node	0.41
			A-58	leaf margin	"
				node	0.42

These two results lead the authors to the conclusion that there is no differences among the several genotypes in regards to the quality of anthocyanin concerned.

II. Further, to clarify an aglucone of this anthocyanin, the colored leaf blade with the genic constitution of C^B Sp Pl was collected at a time slightly before panicle emergence and was ground into flinders. Extracted solutions of 1 % hydrochloric acid were taken from the flinders and were treated according to the qualitative analysis of aglucone offered by Robinson and Robinson (1931). The water solution of aglucone, the resulting hydrolysed substance, shows the following results of examination.

i) The sample shows a violet color when sodium acetate is added to its amyl alcohol extract and ferric chloride changes the said violet to dark blue.

ii) The sample is fairly stable in a solution of 10 % sodium hydroxide.

iii) A small portion of aglucone is extracted when the solution is shaken with an equal volume of a mixture of cyclohexanol (1 vol.) and toluene (5 vols.).

iv) And it is also extracted, to a certain extent, in a 5 % solution with an equal volume of amyl ethyl ether (1 vol.) and anisol (4 vols.).

Identical results are obtained from materials from genotype C^{Bp} Sp Pl, using the same methods.

Therefore, these results seem to indicate that the anthocyanin, which is present in rice plant, and which depends on the multiple allelomorphic series of gene, C and Sp, has cyanidin as its aglucone.

III. For verification of this assumption, the aglucone of anthocyanins extracted from the genotypes of C^B Sp Pl and C^{Bp} Sp Pl were paper-chromatographically identified, and compared with cyanidin specimen, by means of Hayashi and Abe's method (1952). A mixture of iso-amyl alcohol, 36 % hydrochloric acid and distilled water in a ratio of 5:1:1, and Toyo No. 50 filter paper were used for this purpose with the temperature at 25° C.

According to this one dimensional paper chromatography the Rf values of all samples invariably show the same values of 0. 63–0. 64, which are equal to that of the cyanidin specimen; Rf=0. 63.

Considerations

It has been reported that at least one of the aglucones involved in rice anthocyanin is identified as cyanidin by Hayashi (1944). However this study was carried out from chemical point of view, by using purple leaf blades of only one type of coloration in so-called "Murasaki-ine". It is unfortunate that the genic constitution of Hayashi's sample is not ascertained.

The authors have made previous studies on anthocyanins in various parts of rice of several genotypes or genic constitutions with regard to the C-chromogen, Sp-modifier and their distributing genes Rp, Pl and Pn, and determined that these

anthocyanins were the same, with no qualitative differences in spite of numerous variations in color, hue and shade or localization of colors.

And it is emphasized that the aglucone of this anthocyanin was identified to be cyanidin, without exception.

On the whole, therefore, the authors have arrived at the conclusion that the differences of the affection of allelomorphic genes C^B, C^{Bp}, C^{Bl}, C^{Br} anb C^+ are to be regarded as quantitative, and the same should be considered to hold in the case of Sp, Sp^d and Sp^+, though more detailed analysis extending over all genotype combinations would be necessary to determine this opinion conclusively.

Summary

1. The anthocyanin pigments contained in rice plants were analysed by means of paper chromatography, and it was revealed that the said anthocyanins were the same with no qualitative differences within the combinations of color genes, and hues and shades of colors as specified above.

2. The aglucone of this anthocyanin was identified as cyanidin.

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The Filamentous Appendage in the Spermatozoids of Hepaticae as Revealed by the Electron Microscope*

by Syôiti SATô**

佐藤正一**： 苔類精子の繊維状附属物の電子顕微鏡的研究*

It has been previously reported by the author (Sy. Satô 1954)*** that the spermatozoid of *Conocephalum conicum*, a species of Hepaticae, possesses a filamentous appendage which can be seen with the help of the electron microscope. This filamentous appendage is a long tape along the spermatozoid-body, measuring 0.2μ in width. It can not be observed in the specimens fixed with vapor of 2 per cent OsO_4 solution nor with 4 per cent formalin, because it adheres firmly to the spermatozoidbody. In the specimens treated with hot water, ammonium vapor, or ultra-sonics under a living state, the filamentous appendage is exposed from the body of the spermatozoid, and becomes discernible under the electron microscope. In the unfixed specimen, sometimes, the filamentous appendage parts from the body of the spermatozoid thus comes into sight. Thus the filamentous appendage is a fine and delicate substantial which can not be detected by means of the light microscope, but can be seen under the electron microscope.

In the present paper, it will be reported that a similar filamentous appendage which is found in the spermatozoid of *Conocephalum conicum* exists generally in the spermatozoids of many other species of Hepaticae.

Materials and Methods

The following eight species belonging to three orders of Hepaticae were examined :

Metzgeriales :	*Pellia fabbroniana* Raddi
	Makinoa crispata (Steph.) Miyake
Marchantiales :	*Marchantia polymorpha* L.
	Conocephalum supradecompositum (Lindb.) Steph.
	Wiesnerella denudata (Mitt.) Steph.
	Dumortiera hirsuta (Swartz) Reinw. Bl. et N. ab E. subsp. *tatunoi* Horikawa
Anthocerotales :	*Phaeoceros laevis* (L.) Proskauer
	Notothylas japonica Horikawa

The spermatozoids of these liverworts were generally fixed with vapor of 2 per cent OsO₄ solution for the observation of each normal form. In order to examine the presence of the filamentous appendage, the spermatozoids were allowed to dry without fixation, or were treated with hot water. Specimens for the electron microscope were prepared in the usual way by drying a drop of the suspension of the spermatozoids on a collodion film. The shadow casting was done with chromium. The electron microscope employed was a Hitachi HU-4 type installed in the Laboratory of Electron Microscopy of the University of Tokyo.

Results and Discussion

Conocephalum supradecompositum—This species is closely related to *Conocephalum conicum*. It differs from *C. conicum* in having a smaller gametophyte, and it matures in autumn in contrast to the spring for *C. conicum*. The body length of the spermatozoid in these two species differs significantly: that is the body length of *C. supradecompositum* is about half long that of *C. conicum*. A filamentous appendage similar to that observed in the spermatozoid of *C. conicum* was also detected in the spermatozoid of *C. supradecompositum* under the electron microscope in the present study. This filamentous appendage does not appear in such specimens fixed with vapor of OsO₄ solution or formalin, but it can be seen in specimens treated with hot water, because the filamentous appendage attached originally along the spermatozoid-body, remains after the destruction of both nucleic and cytoplasmic portions of the spermatozoid-body with this treatment. The filamentous appendage is not destroyed by hot water, but its original form is preserved.

The filamentous appendage of *C. conicum* was a long tape, about 0.2μ in width, which originates from the blepharoplast and is attached along the spermatozoid-body (nucleic portion and cytoplasmic portion) lengthwise (Fig. 1). The filamentous appendage of *C. supradecompositum* has a similar shape, but a slightly less width, 0.16μ (Fig. 2).

Marchantia polymorpha—The spermatozoids of this species are obtained commonly from spring to autumn in Tokyo. In specimens of the spermatozoids fixed with vapor of 2 per cent OsO₄ solution or with 4 per cent formalin, the filamentous appendage could not be detected. However, when specimens were treated with hot water, the filamentous appendage exposed itself clearly and remained after the nucleic and cytoplasmic portions were dispersed by hot water. This filamentous appendage is also a long tape, about 0.2μ in width like *C. conicum* (Fig. 3).

Wiesnerella denudata—The spermatozoids of this species mature in spring. In specimens fixed with vapor of 2 per cent OsO₄ solution or with 4 per cent formalin the filamentous appendage does not appear as in *Marchantia polymorpha*, but, it becomes visible after the nucleic portion has swelled and is destroyed naturally in the unfixed specimens. This filamentous appendage is also a long tape, about 0.25μ in width (Fig. 4).

Dumortiera hirsuta subsp. *tatunoi*—The present sample was an intraspecific polyploid of *Dumortiera hirsuta*, triploid (n=27), collected by the present author at Zinmuzi in Miura Peninsula and determined by Dr. S. Hattori. In the spermatozoids of this triploid plant are there two types; one has two flagella, and the other has four flagella. Irrespective of the types, each spermatozoid had a long tape like filamentous appendage, about $0.25–0.35\mu$ in width (Figs. 8 and 9). The spermatozoids of liverworts have generally two flagella, so that it is interesting that *D. hirsuta* subsp. *tatunoi* produces two types of spermatozoids as above mentioned. The filametous appendage is one in both spermatozoids. Comparative morphological studies of the spermatozoids in triploid plant as well as that of haploid and diploid plants will be reported elsewhere.

Makinoa crispata—The spermatozoid of this species is the largest one in liverworts, having 70μ in body-length. This filamentous appendage is a long tape, about $1–1.2\ \mu$ in width (Fig. 5). Fig. 5 shows a part of a spermatozoid near the blepharoplast, and shows that the filamentous appendage originates from the blepharoplast and separates from the spermatozoid-body by chance. It was found that the filamentous appendage of this plant is wider than the nucleic portion near the blepharoplast, and the filamentous appendage is very thin.

Pellia fabbroniana—The spermatozoid of this species belongs also to the largest in liverworts such as *Makinoa crispata*. It is about 70μ in body-length. It seems that this filamentous appendage does not separate easily from the body of the spermatozoid. Fig. 6 shows that a filamentous appendage separated partially from the body. In the specimens treated with hot water, the width of the filamentous appendage was about 0.4μ. This is small exceedingly as compared with that of *Makinoa crispata*.

Notothylas japonica and *Phaeoceros laevis*—The form of the spermatozoids of Anthocerotales is very different from those of the other orders, Marchantiales and Metzgeriales. The spermatozoid-bodies of *N. japonica* and *P. laevis* show a long dumb-bell shape, and each nucleic portion has a slender portion which scarcely stains with Feulgen reaction or Heidenhain's iron alum hematoxylin. The nucleic portion attaches a blepharoplast to its front part, and attaches a cytoplasmic portion to its posterior respectively. The filamentous appendage exists in both *N. japonica* and *P. laevis*, and the form is tape like, about 0.12μ in *N. japonica* (Fig. 7) and about 0.08μ in *P. laevis*.

The spermatozoids of nine species of three orders in Hepaticae have been observed by means of the electron microscope. And it becomes clear that the filamentous appendage exists in the spermatozoids of liverworts without exception so far, and that it originates in the blepharoplast streching along the nucleic and cytoplasmic portions forming a long tape. Previously it was found by the present author that the filamentous appendage of the spermatozoid of *C. conicum* is composed of ten fine fibrils (unpublished). In the present study it was also found that

the filamentous appendage of other species among liverworts most probably consists of many fibrils, the number of which could not be confirmed yet.

It is obscure as yet what function the filamentous appendage has. This structure may serve to support the structure of the spermatozoid-body.

The chemical differences between the filamentous appendage and flagellum of the spermatozoid of *C. conicum* have not yet been made clear, but morphologically from the electron microscopical observations, the flagellum is composed of eleven fibrils forming a cylindrical array, and the filamentous appendage is composed of ten fibrils which keep side by side in a plane. The width of the fibrils of the filamentous appendage differs significantly from that of the flagellum. This will be explained in the future. It is a question whether the presence of the filamentous appendage is specific to the spermatozoids of liverworts or universal in plants, mosses and ferns. This is now under investigation.

Summary

The filamentous appendage similar to that found in the spermatozoid of *Conocephalum conicum* has been confirmed in the following eight species belonging to three orders of Hepaticae: Metzgeriales: *Pellia fabbroniana, Makinoa crispata,* Marchantiales: *Marchantia polymorpha, Conocephalum supradecompositum, Wiesnerella denudata, Dumortiera hirsuta* subsp. *tatunoi,* Anthocerotales: *Phaeoceros laevis, Notothylas japonica.*

Explanation of Plates

Electron micrographs of spermatozoids of Hepaticae. Chromium shadowed. A: Filamentous appendage, B: Flagellum, C: Spermatozoid-body. Scale shows 1μ. Plate IX. Fig. 1. *Conocephalum conicum.* Treated with hot water. A filamentous appendage(A) remained along the spermatozoid-body(C) which dispersed due to the treatment of hot water. Fig. 2. *Conocephalum supradecompositum.* Unfixed specimen. When the spermatozoid is allowed to dry without fixation, it is destroyed partly, and a filamentous appendage separates from the body. Fig. 3. *Marchantia polymorpha.* Treated with hot water. Fig. 4. *Wiesnerella denudata.* Unfixed specimen. Plate X. Fig. 5. *Makinoa crispata.* Unfixed specimen. Part of a spermatozoid near the blepharoplast, showing a wide tape like filamentous appendage(A). Fig. 6. *Pellia fabbroniana.* Unfixed specimen. Part of a spermatozoid does not separate completely from the body, seen as the fin. Fig. 7. *Notothylas japonica.* Unfixed specimen. Showing a body of a dumb-bell like shape and a filamentous appendage(A). Figs. 8 and 9. *Dumortiera hirsuta* subsp. *tatunoi.* Treated with hot water. Fig. 8 shows the spermatozoid having two flagella attached to it, and Fig. 9 shows the spermatozoid having four flagella. Each has a filamentous appendage.
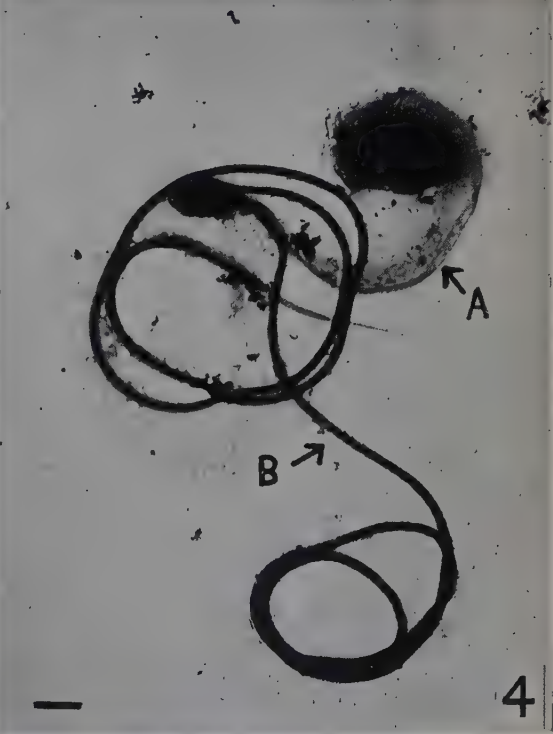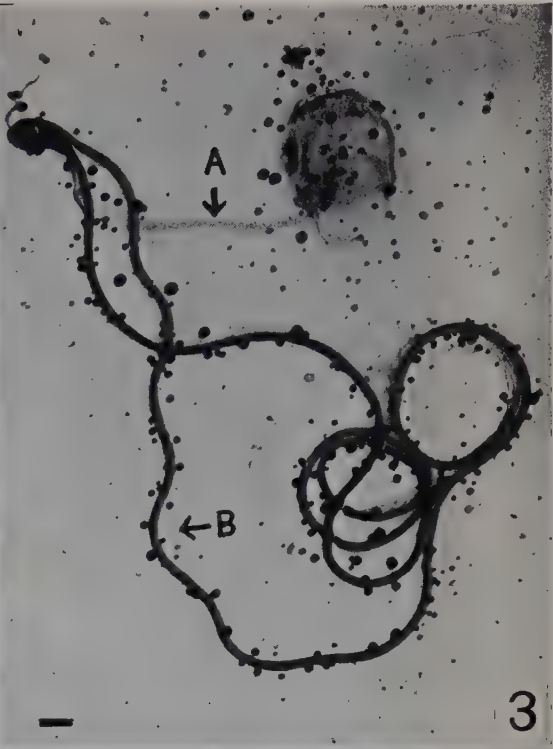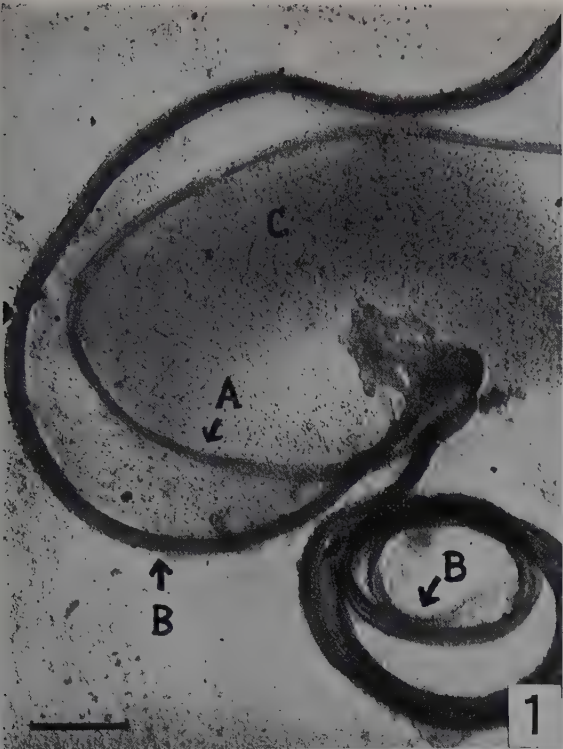
Sy. Satô: Filamentous appendage in the spermatozoids of Hepaticae.
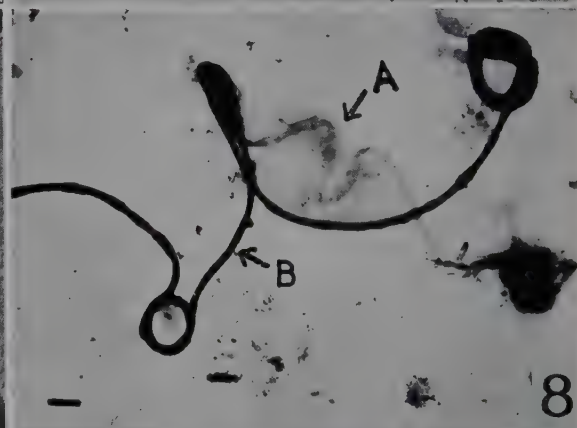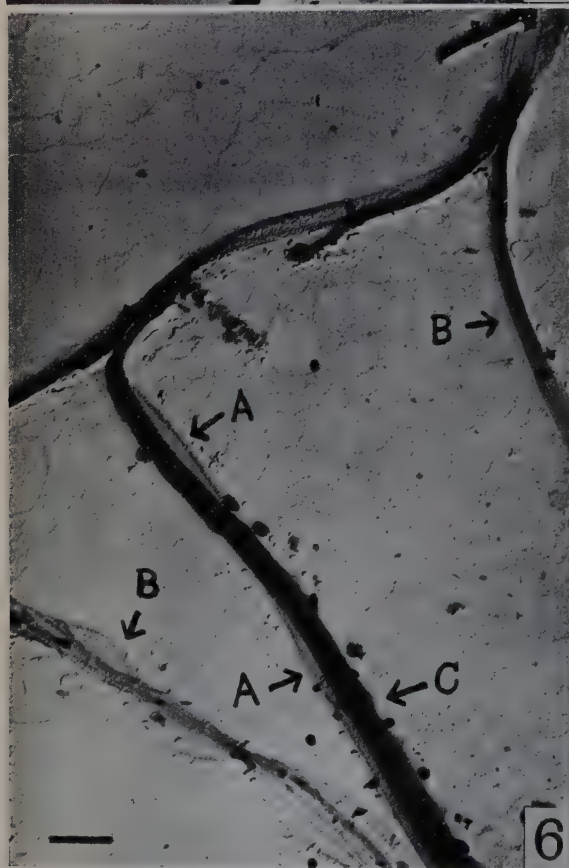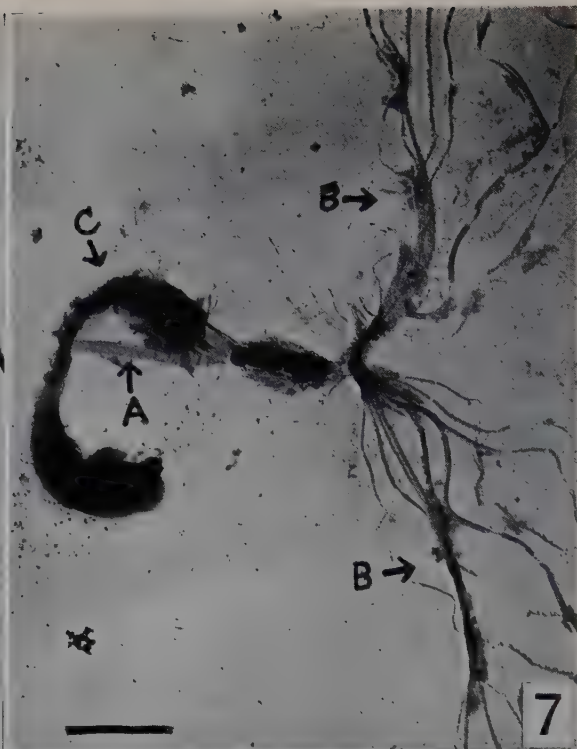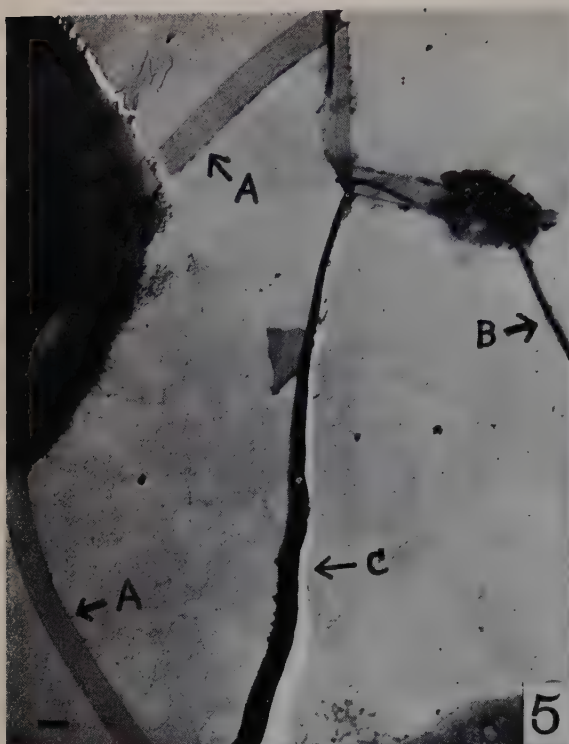
Sy. Satô: Filamentous appendage in the spermatozoids of Hepaticae.

The Embryogeny of *Cupressus funebris* Endlicher

by Yosinori SUGIHARA*

杉原美徳*: シダレイトスギの胚発生

In the previous report (Sugihara, 1956), it was described that the embryogeny of *Cupressus sempervirens* L., the type species of the genus *Cupressus*, is a new type in the conifers. From a study on *Cupressus funebris* it was found that its embryogeny decidedly differs from that of *C. sempervirens*, previously studied.

The material used in the present work was collected from a tree cultivated in the Experimental Forest of the University of Tokyo at Amatsu, Chiba Prefecture. The technique used in this study is the same as that employed in the previous work.

The archegonia are grouped together in a complex manner at the apex of the prothallium. On the outside of the archegonial complex a single layer of jacket cells is found. Such a common jacket layer is usually found in *Chamaecyparis* (Sugihara, 1938), but is not formed in *Cupressus sempervirens* (Sugihara, 1956). On the outside of each archegonium in *C. sempervirens* there is found a layer of thick wall, but in *C. funebris* as in *Chamaecyparis* such a thick wall is not formed.

The fertilization takes place in the middle of July. The contact of the male and female nucleus takes place at the upper part of the archegonium (Fig. 1). The female nucleus is distinctly larger than that of the male. They are wrapped in a coarse granular cytoplasm. The fertilized nucleus soon divides into two nuclei (Fig. 2). Both daughter nuclei divide synchronously twice to form eight free nuclei (Fig. 3). Then, the first wall formation takes place in the proembryo, thus the proembryonal free nucleate condition comes to an end at the eight nucleate stage. The resulting eight cells are disposed in two tiers (Fig. 4), of which those in the upper tier have no wall towards the archegonial cavity. In the next stage the cells of such proembryo divide into 16 cells and they are disposed into three tiers (Fig. 5). The uppermost tier is the open cell tier, and degenerates to a later stage while the middle tier of 4 to 6 cells, elongates to form the prosuspensor and the lowest tier with 6 to 8 cells, is the embryonic tier. At this stage the proembryonal development comes to an end. Then, the prosuspensor begins to elongate (Figs. 6-8). After considerable elongation of the prosuspensor each units at the tip of the prosuspensor divides transversely into two cells (Fig. 9). Of the two daughter cells the one which lies next to the prosuspensor begins to elongate to form the primary

* Department of Biology, Faculty of Education, Tôhoku University, Sendai. 東北大学教育教養部
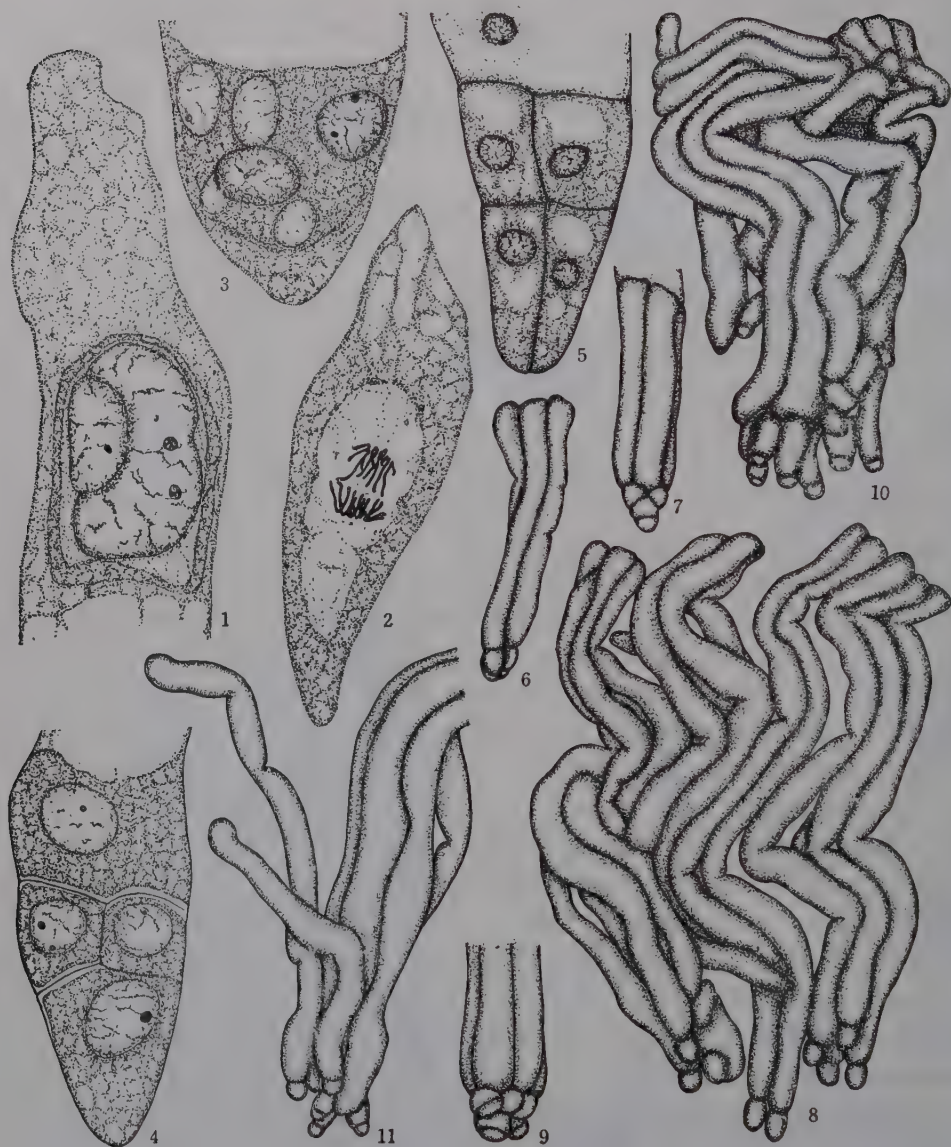
Figs. 1-11. Fertilization and embryo in *Cupressus funebris*.

1, Fertilization (× 600). 2, Proembryonal first mitosis in anaphase (× 600). 3, Eight
nucleus stage of a proembryo (× 600). 4, Eight cell stage of a proembryo (× 600).
5, 16 cell stage of a proembryo (× 600). 6-7, Early stage of the prosuspensor elongation
(× 120). 8, More advanced stage of the prosuspensor elongation (× 120). 9, Embryonic
units divided into two cells at the tip of a prosuspensor (× 120). 10, Beginning of the
elongation of the primary suspensor (× 120). 11, More advanced stage of the primary
suspensor elongation (× 120).

suspensor (Figs. 10–11). As the result of this, the cleavage polyembryony takes place at the tip of the prosuspensor. The prosuspensor elongates not so long and the tip of it seems to become separated into individual prosuspensor cells. After the primary suspensor considerably elongates, it becomes separated easily from the tip of the prosuspensor. From the foregoing it may be seen that the development of the embryo in *Cupressus funebris* is identical with that of *Chamaecyparis* (Buchholz, 1932; Sugihara, 1938), but not with that of *Cupressus sempervirens* (Sugihara, 1956). Lawson (1907) states that in *Cupressus* sp. the proembryo of eight free nuclei is formed and Doak (1937) described that in *Cupressus arizonica* the proembryo of 16 cells is found. However, it seems to the present writer that their works on the proembryo reserve more room for study.

In some species of *Cupressus* as, *C. Goweniana* (Juel, 1904), *C. arizonica* (Doak, 1932), and *C. sempervirens* (Sugihara, MS), a large number of male gametes are formed in a pollen tube. But in *C. funebris* (Mehra and Sircar, 1949; Sugihara, MS) only two gametes are formed in a pollen tube as is found in *Chamaecyparis* (Sugihara, 1938). Some external characteristics of *C. funebris* are identical with those of the other species in *Cupressus*, but in certain other features with that of *Chamaecyparis*.

In conclusion, it is to be said that *Cupressus funebris* is an exception in the genus *Cupressus*. If it is to be held that embryology is taxonomically important, it may be suggested that the systematic position of *Cupressus funebris* should be reconsidered. If may also be added that, from the results of embrylological study, *C. funebris* should be transferred from *Cupressus* to *Chamaecyparis*. However, until embryological studies of the other species of *Cupressus* have been undertaken definite conclusions must be reserved.

Here the writer wishes to express his hearty thanks to Profs. T. Inokuma and S. Takahara and Assist. Prof. S. Watanabe, for their kindness during the writer's collecting in the field.

Summary

In the proembryo of *Cupressus funebris* eight free nuclei are formed and then the first wall formation takes place. In the 16 cell stage, the proembryonal development comes to an end. The cleavage polyembryony is usually found. The embryogeny of *C. funebris* is identical with that of *Chamaecyparis*, but not with that of *C. sempervirens*.

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Development of the Leaf Margin in the Ericaceae*
(A Preliminary Report)

by Noboru Hara**

原 　裏** ツツジ科の葉縁の発達(予 報)

According to Solereder(1899), it has been evidently observed by Ljungström(1883) that the revolute leaf in *Erica* is formed, not by the revolution of the margins of the lamina, but by the formation of two coherent pads along the margins of the lamina on the lower side of the leaf. Hagerup(1953) has recognized three fundamental types (the Rododendron leaf, the needle-shaped leaf, and the ericoid leaf) in the forms and the structures of the leaves in the Ericales, on the basis of the features of development of the leaves mentioned above. He emphasizes that the features of leaves in the Ericales are to be more sufficiently utilized in systematics and suggests that only the species which have the ericoid leaves should be placed in one independent family, that is, Ericaceae proper.

During the course of a phylogenetical study of the Ericales on the basis of an extensive observation on the development of the foliage shoot, the writer arrived, in the explanation on the manner of the development of the foliage leaves, at somewhat different results from Hagerup's observation. The following brief report is referred to this problem.

A   **B**
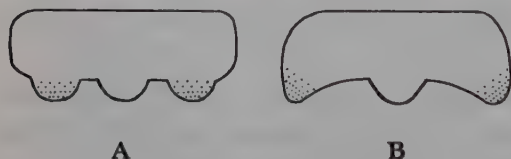
Fig. 1: Diagrams of the ericoid leaf (A), and the rolled leaf (B). Dotted parts develop in future as the leaf margins.

A   **B**

Fig. 2: Diagrams of the revolute leaf (A), and the convolute leaf (B). Dotted parts develop in future as the leaf margins.

It is appropriate for convenience that we apply the term *ericoid* leaf to the one which protrudes two pads on its lower side (Fig 1. A), and the term *rolled* leaf to the one which rolls up its margins toward its lower side (Fig. 1. B). The term *revolute* leaf is also used, regardless to the manner of the development, to the leaf with the revolute margins, including both of the ericoid and the rolled leaves (Fig. 2. A), and the term *convolute* leaf to the one whose margins are convolute (Fig. 2. B).

Materials and Methods —Most materials were collected at the Nikko Branch, Botanic Gardens of University of Tokyo, Mt. Taro in Nikko, Mt. Hakkoda, and Mt. Nishikoma. *Tsusiophyllum Tanakae* was collected by Mr. H. Kanai at Mt. Ashitaka and Mt. Kinpu.

The species used in the present study are as follows.

Rhododendroideae: *Daboecia cantabilina* Koch; *Ledum palustre* L.; *Loiseleuria procumbens* Desvaux; *Menziesia ciliicalyx* Maxim., *M. pentandra* Maxim.; *Phyllodoce aleutica* A. Heller, *P. nipponica* Makino; *Rhododendron Degronianum* Carrière, *R. japonicum* Suringar, *R. Kaempferi* Planchon, *R. Keiskei* Miquel, *R. indicum* Sweet, *R. macrosepalum* Maxim., *R. pulchrum* Sweet, *R. quinquefolium* Bisset et Moore, *R. semibarbatum* Maxim., *R. Tschonoskii* Maxim., *R. Wadanum* Makino; *Tripetaleia bracteata* Maxim., *T. paniculata* Sieb. et Zucc.; *Tsusiophyllum Tanakae* Maxim.

Arbutoideae: *Andromeda Polifolia* L.; *Arcterica nana* Makino; *Arctous alpinus* Niedenzu; *Cassiope lycopodioides* D. Don; *Chiogenes japonica* A. Gray; *Enkianthus perulatus* Schneider; *Epigaea asiatica* Maxim.; *Eubotryoides Grayana* Hara; *Gaultheria adenothrix* Maxim., *G. Miqueliana* Takeda; *Harrimanella Stelleriana* Coville; *Leucothoe Keiskei* Miquel; *Lyonia Neziki* Nakai et Hara; *Pieris japonica* D. Don; *Tritomodon campanulatus* F. Maekawa.

Vaccinioideae: *Oxycoccus quadripetalus* Gilibert; *Vaccinium Usunoki* Nakai, *V. Vitis-Idaea* L.

Ericoideae: *Erica tetralix* L.

Materials were fixed in FAA or Craf II, and dehydrated in normal butyl alcohol. Paraffin sections were usually cut at 10μ, and stained with safranin and haematoxylin or fastgreen.

Observation

Rhododendroideae.—*Tripetaleia.* In *T. bracteata* and *T. paniculata*, leaves are not revolute but show the convolute type throughout all stages of their development (Plate XI, Fig. 3).

Phyllodoce. Leaves of *P. aleutica* and *P. nipponica* (Plate XI, Figs. 1. 2) show the typical ericoid type, as has been already observed by Hagerup. The periclinal divisions in the subepidermal cells of the lower side of the leaf are observed obviously in very earlier stages of the development, resulting the formation of the pads (Plate XI, Fig. 2). As shown in the plate, prominent glandular hairs are formed on true margins of the leaf. It may be able to recognize the relation between the formation of the glandular hairs and the declining activity of the submarginal initials of the ture margin of the leaf.

Daboecia. The leaf of *D. cantabilina* assumes the type of the rolled leaf in its earlier stages of development. But, it can be observed that periclinal cell divisions in the lower side of the leaf as in the case of *Phyllodoce* appear in the first stage of development of the margins in which the rolled parts begin to project from a some-

what flattened cylindrical primordial leaf, and it is certain that the derivatives from these divisions become the future margins of this leaf (Plate XI, Fig. 4). Thus, the development of the margins is obviously of the ericoid type, and in the later stages these margins develop so as to form the normal rolled leaf. After the leaf expands broadly in the matured stage, margins are also slightly revolute, but there remains no trace of the ericoid type.

Loiseleuria. In *L. procumbens*, the leaf shows the rolled type throughout all stages of its development. It does not expand in its matured stage, but maintains the rolling (Plate XI, Fig. 5, two central leaves).

Menziesia. The development of the leaf of *M. pentandra* assumes generally the type of rolled leaf. However, when glandular hairs emerge on its margin in the earlier stages of its development, the periclinal divisions are apt to occur in cells of the lower subepidemal layer which are not the true submarginal initials (Plate XI, Fig. 6). Thus, it is certainly sure that the derivatives become new submarginal cells and develop hereafter toward the lower side of the leaf to form the rolled part. This relation of the periclinal divisions in cells of the lower subepidermal layer to the glandular hairs resembles to the case of *Phyllodoce*. In the leaf of *M. pentandra* the same periclinal divisions are also observed frequently in the place of no indication of the glandular hair. The development of the leaf of *M. ciliicalyx* is almost the same as in the case of the former species. In the bud the young leaves are to some extents revolute, but gradually lose this feature in the later stages.

Tsusiophyllum. The process of the leaf development in *T. Tanakae* is almost the same as in the case of *Menziesia* (Plate XII, Fig. 1).

Rhododendron. The development of the leaf of *Rhododendron* assumes generally the type of the rolled leaf (Plate XII, Fig. 2). The periclinal divisions in the same position such as in *Menziesia* and *Tsusiophyllum* can be observed (Plate XII, Fig. 3). In *Rhododendron*, however, it is generally difficult to distinguish whether the derivatives of these divisions become functionally to the new submarginal initials or become merely to the mesophyll cells. In the bud the young leaves are to some extents revolute, but usually lose this feature gradually in the later stages of their development, though in a certain species they do not always lose their rolled feature in the matured stage. For example, the leaf of *R. Degronianum* strongly shows the revolute form in winter. The extremely rolled leaves in the bud are observed in *R. semibarbatum* (Plate XII, Fig. 4).

R. Keiskei is an aberrant among the species of this genus in that the leaf is not revolute but shows the convolute type in its bud, though the margins of the leaf in the bud recurve very slightly toward the lower side (Plate XII, Fig. 5).

Ledum. As is shown in *Rhododendron*, the development of leaf of *L. palustre* assumes the type of rolled leaf, and the same periclinal divisions such as in the case of *Rhododendron* can also be observed here. The matured leaf is slightly revolute.

Arbutoideae and Vaccinioideae. The leaf margin in these subfamilies is not

revolute in the earlier stages of its development, but generally shows the convolute type, with only an exception of *Andromeda Polifolia* whose development of the leaf is almost the same with *Ledum palustre* (Plate XII, Fig. 6).

The species, which show the convolute leaves in the earlier stages of development, are observed in the following genera, that is, *Arcterica**, *Arctous, Cassiope, Chiogenes, Enkianthus, Epigaea***, *Eubotryoides, Gaultheria, Harrimanella, Leucothoe, Lyonia, Pieris, Tritomodon, Oxycoccus*, and *Vaccinium*. The matured leaves of these genera also are not revolute commonly, but there are some species, whose leaves assume more or less the appearance of the revolute feature. They are *Arcterica nana, Oxycoccus quadripetalus*, and *Vaccinium Vitis-Idaea*. Furthermore, the leaf of *Cassiope lycopodioides* shows a curious form in its matured stage (cf. Solereder, 1899).

Ericoideae. The leaf of *Erica tetralix* shows the ericoid type, as has already been observed by Ljungström, etc.

Conclusive Summary

On the basis of the development of leaf margin, Hagerup considers that *Phyllodoce* should be separated from the other members of the Rhododendroideae, since *Phyllodoce* shows a form of the ericoid leaf, while others have flat leaves and if they have recurved leaves, they are true rolled leaves. He notes a close relationship among *Phyllodoce, Erica*, and *Empetrum*, in having the typical ericoid leaves. It is, however, to be noticed that, in the present study, there are found some evidences of the ericoid type in the development of leaves in *Daboecia, Menziesia, Tsusiophyllum, Rhododendron*, and *Ledum*, in which the periclinal divisions occur frequently, in the earlier stages of the leaf development, in the cells of the lower subepidermal layer which are not the true submarginal initials and it is certainly sure in the most cases that the derivatives become new submarginal initials which form the rolled part of the leaf. In this connection, it may be recognizable that there is a certain relationship among *Phyllodoce, Erica*, and *Empetrum* as was accepted by Hagerup, but it can also be thought that *Phyllodoce* shows some close relation to the genera of the same subfamily, such as *Menziesia, Tsusiophyllum, Rhododendron*, and *Ledum*. These relationships probably involve some significant problems from the evolutional standpoint.

Thus, so far as the present preliminary observation is concerned, the writer should like to conclude that it is only possible to distinguish the following two types in regard to the manner of the development of the leaf margin, that is;

I. Revolute Type (Fig. 2, A), in which the leaf is revolute in the earlier stages of its development.

II. Convolute Type (Fig. 2, B), in which the leaf is not revolute, but convolute in the earlier stages of its development.

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*, **: These leaves show slight indications of the revolute feature in their budal stage.

The revolute type includes the leaves of *Daboecia, Ledum, Loiseleuria, Menziesia, Phyllodoce,* most species in *Rhododendron, Tsusiophyllum, Andromeda,* and *Erica.* The convolute type includes the leaves of a few species in *Rhododendron, Tripetaleia, Arcterica, Arctous, Cassiope, Chiogenes, Enkianthus, Epigaea, Eubotryoides, Gaultheria, Harrimanella, Leucothoe, Lyonia, Pieris, Oxycoccus,* and *Vaccinium.*

As mentioned above, it is particularly important that there are no decided distinction between the *ericoid* and the *rolled* leaves at certain earlier stages of their development, or, in other words, both types are derived only by the slight differences of manner in their further development.

Furthermore, it is very interesting that the leaves of *Tripetaleia* and *Rhododendron Keiskei* show the convolute type, regardless the leaves of most species in the Rhododendroideae show the revolute type. *Tripetaleia* has been considered, on the one hand, to be more primitive genus within the Rhododendroideae by Copeland (1943), and Matthews and Taylor (1926), etc., while, on the other hand, Sinclair (1937) has considered that *Lepidote* series characterized by the convolute type of bud is a progressive one within *Rhododendron,* which is commonly considered to be more progressive genus within the Rhododendroideae. But, *R. Keiskei* is not included in this series. In this regard, this species proposes a very interesting problem on the phylogenetic relationships of the *Rhododendron.*

At any rate, further critical studies are desirable for the diverse forms of the leaves in the Ericaceae, so that further studies will be carried on in detail by the present writer.

The author wishes to express his best thanks to Prof. Y. Ogura and Dr. S. Watari for their kind direction and advice. His thanks are also due to Mr. S. Nakamura of the Nikko Branch, Botanic Gardens of University of Tokyo, and Mr. H. Kanai for valuable materials.

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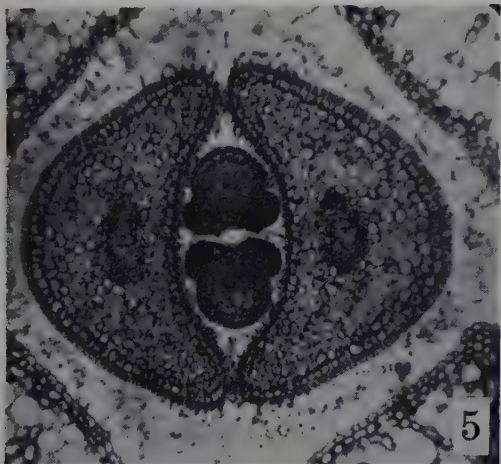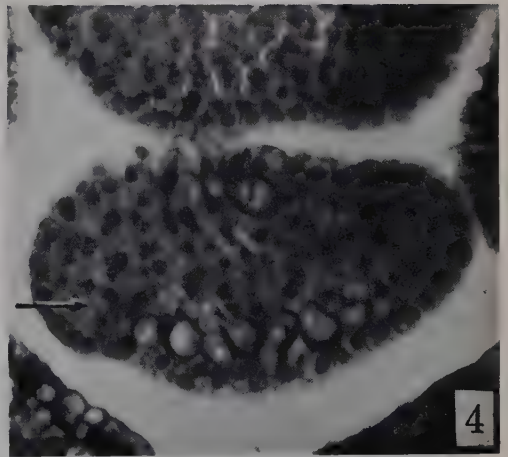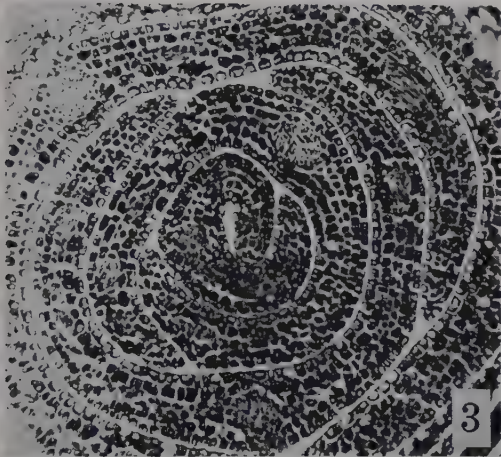
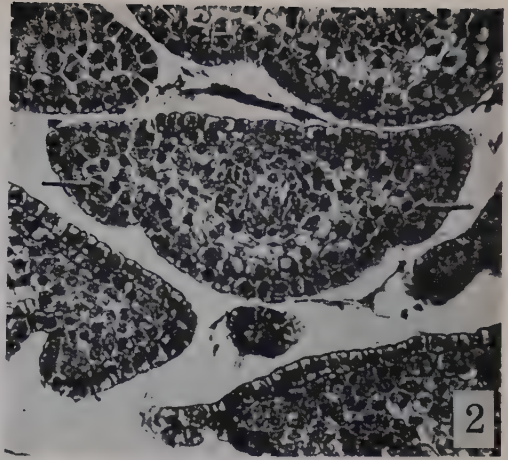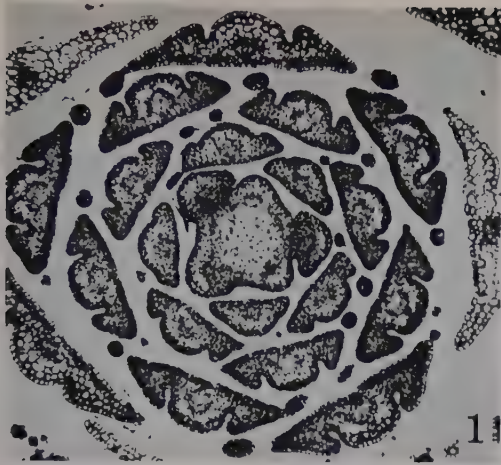Plate XI, Fig. 1: *Phyllodoce nipponica*, with glandular hairs, ×70, Fig. 2: *Phyllodoce nipponica*, with glandular hairs, ×300, Fig. 3: *Tripetaleia paniculata*, ×100, Fig. 4: *Daboecia cantabilina*, ×600, Fig. 5: *Loiseleuria procumbens*, surrounded with two petioles, ×100, Fig. 6: *Menziesia pentandra*, with glandular hairs, ×800. Upper side of the solitary leaf (Figs. 2, 4, 6) is shown in the upper side of the figure. Arrows show the portions showing the periclinal divisions in the cells of the lower side of the leaves.

N. Hara: Development of the leaf margin in the Ericaceae.
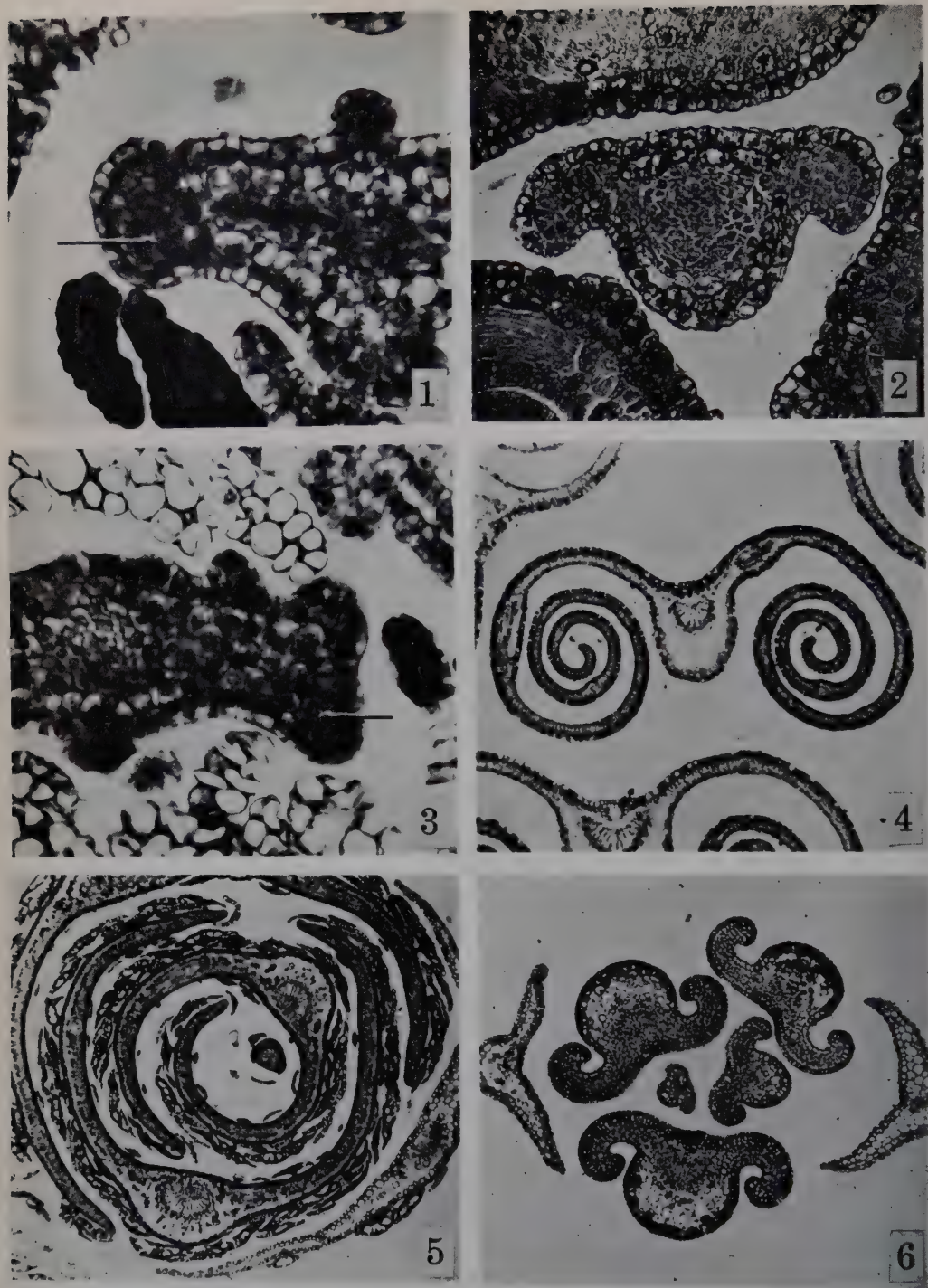
Plate XII, Fig. 1: *Tsusiophyllum Tanakae*, with glandular hairs, ×600, Fig. 2: *Rhododendron japonicum*, ×150, Fig. 3: *Rhododendron Tschonoskii*, ×450, Fig. 4: *Rhododendron semibarbatum*, ×40, Fig. 5: *Rhododendron Keiskei* with scaly hairs on abaxial surfaces, ×40, Fig. 6: *Andromeda Polifolia*, with two scale leaves, ×60. Upper side of the solitary leaf (Figs. 1, 2, 3, 4) is shown in the upper side of the figure. Arrows show the portions showing the periclinal divisions in the cells of the lower side of the leaves.

N. Hara: Development of the leaf margin in the Ericaceae.

Remains of *Pinus koraiensis* S. et Z. and Assosiated Remains in Japan

by Shigeru MIKI*

三木　茂*：　チョウセンゴョウ遺体並びにその共存植物遺体について

Distribution of living species of *Pinus koraiensis* S. et Z. is restricted to the mountainous regions of Japan, northern Korea and eastern Manchuria in Asia. Growing sites in Japan are found scattered in the foggy regions 1050 m–2600 m above sea-level in Central Honshu (1) and one place in Shikoku (10).

The remains were first discovered in the conifer bed in Egota, Tokyo City (5), next in the *Larix* bed in Manzidani, Nishinomiya City (6) and in many places in a wide area as shown by following data (9). It is usually associated with many subalpine conifers, growing on mountains of Central Honshu, about 1500 m above sea-level.

The localities, associated remains, age of migration and depression of temperature of the Pleistocene age are discussed in this paper.

Remains

The cones, leaves and seeds were found in the remains of *Pinus koraiensis*. The seed remains were excavated especially rich in durable testa and easily identified by many special characteristics viz. obovate shape with a sharp ridge reduced from a wing at the upper margin and hilum near the apex of the dorsal side. The structure of the testa is composed of sclerenchymatous cell, but not of pallisade tissue.

The remains were found in 24 different localities as in Fig. 1 and Table 1.

The altitude of the localities is distributed from 20 m to 500 m above sea-level, but there is a distinct difference between the altitude of the present growing places and the localities of past remains.

Associated floral remains

About 55 species of floral remains belonging to 35 genera and 22 families, associated with *Pinus koraiensis* have been found.

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* Institute of Polytechnics, Osaka City University, Osaka, Japan.　大阪市立大学理工学部

Table 1. Localities of the remains of *Pinus koraiensis* S. et Z.

Localities (altitude)	Remains	Associated remains	
		No. of species	Representative species
1) Mammalian bed of Kanamori in Hanaidzumi, Iwate Pref. (38 m) (by N. Naora)	Seeds (56)	4	*Larix dahurica* var., *Picea glehni, Corylus sieboldiana*
2) Peat bed near Teramae, Okoe-mura, Tamura-gun, Fukushima Pref. (450 m)	Seeds (2)	3	*Larix leptolepis, Picea glehni*
3) Jigozi of Motohatiozi-mura, Minami Tama-gun, Tokyo Pref. (180 m) (by S. Kuwano)	Leaves	6	*Abies homolepis, Picea yezoensis* var., *Tsuga sieboldii*
4) Conifer bed of Egota, Tokyo City (38 m)	Seeds (13) Leaves	23	*Abies homolepis, Larix leptolepis, Picea glehni, Picea maximowiczii, Picea yezoensis* var., *Picea bicolor, Tsuga diversifolia*
5) Clay bed of Uwano, Tonami-gun, Toyama Pref. (40 m)	Seeds (5)	23	*Abies homolepis, Picea bicolor* (Pl. Aa), *Picea yezoensis* var. (Pl. Ca), *Tsuga diversifolia*
6) Upper bed of *Pinus trifolia* bed in Hara, Turuoka, Gifu Pref. (500 m)	Cone Seeds (11) Leaves	6	*Abies veitchii* (Pl. Da), *Larix leptolepis* (Pl. Hb), *Picea koyamai* (Pl. Ba), *Tsuga diversifolia*
7) Upper bed of *Metasequoia* bed in Daimon, Tado, Mie Pref. (60 m)	Seed (1) Leaves	19	*Abies veitchii, Abies homolepis, Larix leptolepis* (Pl. Ha), *Picea bicolor, Tsuga sieboldii* (Pl. Ka)
8) Upper bed of *Metasequoia* bed in Kodzuhata, Gamo-gun, Shiga Pref. (300 m)	Seeds (3)	11	*Abies homolepis* (Pl. Ea–b), *Picea koyamai* (Pl. Bb), *Pinus parviflora* (Pl. G), *Tsuga sieboldii* (Pl. Kb)
9) Clay bed of Higashiaioi, Obama City, Fukui Pref. (60 m)	Seeds (13)	4	*Alnus hirsuta, Corylus sieboldiana, Sambucus sieboldiana*
10) Cliff of school in Kamiyamada, Yosa-gun, Kyoto Pref. (20 m)	Seeds (33) Leaves	6	*Abies veitchii, Picea bicolor, Picea yezoensis* var., *Tsuga sieboldii*
11) Peat bed of Tengawa, Hieta-mura, Kyoto Pref. (120 m)	Leaves	9	*Abies veitchii, Picea bicolor, Molinia japonica*
12) Peat bed in Otuti-Pass, Togo-mura, Toyono-gun, Osaka Pref. (300 m)	Seeds (4) Leaves	10	*Abies homolepis, Picea maximowiczii, Picea yezoensis* var., *Tsuga diversifolia* (Pl. J), *Betula ermani* (Pl. M)
13) West valley of Kantengoya, Nishinomiya City (by S. Kokawa) (250 m)	Seeds (5)	8	*Metasequoia disticha, Picea maximowiczii, Thuja protojaponica* (Pl. I), *Chamaecyparis obtusa, C. pisifera*
14) Peat bed of Manzidani, Nishinomiya City, Hyogo Pref. (45 m)	Seeds (139)	14	*Abies veitchii* (Pl. Db), *Chamaecyparis pisifera, Larix leptolepis, Picea bicolor, Picea maximowiczii, Thuja protojaponica, Betula platyphylla* (Pl. Nc)
15) Cutting of Horaiso, Arima, Kobe City (400 m), Hyogo Pref.	Seeds (2)	4	*Tsuga sieboldii, Viburnum furcatum*
16) Clay bed of west place in Manganji, Tada, Hyogo Pref. (160 m)	Seeds (5)	1	
17) Basal clay of pond in Higashinamura, Hojio-tyo, Hyogo Pref. (60 m)	Seed (1) Leaves	4	*Abies homolepis, Picea bicolor, Pterocarya rhoifolia*

18) Clay bed of Kadzuwa, Nada-temura, Tohaku-gun, Tottori Pref. (60 m)	Seeds (3)	6	*Picea bicolor, Corylus hetero-phylla, Alnus hirsuta* var.
19) River side peat bed of Gan-manzi, Miyoshi-tyo, Hiroshi-ma Pref. (155 m)	Seeds (7)	5	*Picea bicolor* (Pl. Ab), *Prunus salicina, Alnus hirsuta* var.
20) Peat bed in Futakami of Gota-mura, Kamo-gun, Hiro-shima Pref. (240 m)	Seeds (2)	13	*Picea maximowiczii, Chamaecy-paris obtusa, Styrax obassia, Me-nyanthes trifoliata*
21) Peat bed of Takadzu, Yama-gun, Shimane Pref. (220 m)	Seeds (10)	5	*Abies veitchii, Picea yezoensis* var. (Pl. Cb), *Chamaecyparis obtusa, Tsuga sieboldii*
22) Clay bed in Pass of Kami-sohara, Mine City, Yama-guti Pref. (140 m)	Seeds (10) (Pl. Fa) Leaves	7	*Abies homolepis* (Pl. Ec), *Pinus parviflora, Tsuga sieboldii, Betula grossa, Carpinus erosa*
23) Clay bed of Mt. Terao in Kuma-tyo, Kamiukena-gun, Ehime Pref. (500 m)	Seed (1) Leaves	5	*Abies veitchii, Picea yezoensis* var., *Alnus hirsuta* var. (Pl. L), *Tsuga diversifolia*
24) School side clay bed in Ino-tuki, Emukai-tyo, Nagasaki Pref. (100 m)	Seeds (6) (Pl. Fb)	6	*Abies homolepis, Picea yezoensis* var., *Alnus hirsuta, Betula pla-typhylla* (Pl. Na–b)

There is a wide range of coexistent species; that is: many species were found in one or two places, and 10 species were found in more than five localities. In this paper the latter was named as domi-nant species, most of which grew in the mountain region of Central Honshu, Japan.

Following species are new records for the discovery of the remains in Japan; *Betula ermani, Betula platyphylla, Larix dahurica* var., *Picea glehni, Picea koyamai* and *Pinus parviflora.*

Pinus koraiensis coexists with *Metasequoia, Thuja pro-tojaponica, Pterocarya paliurus* belonging to the elements of the Pliocene and was found only in Kantengoya which lies near Mt. Rokko.

Fig. 1. Map showing the localities of the remains (black) and living one (dotted) of *Pinus koraiensis* S. et Z. Localities of living one were cited from Y. Hayashi (1).

Geological age

The conifer bed of Egota (=Ekoda) is considered as the upper Pleistocene by Yabe (11) etc., and the *Larix* bed of Manzidani as the middle Pleistocene by Huzita (2),

Table 2. Associated remains and their localities

Associated remains	No. of localities	Associated remains	No. of localities
Coniferae		*Carpinus erosa* Bl.	4, 7, 22
Abies homolepis S. et Z.	3, 4, 5, 7, 8, 12, 17, 22, 24	*Cercidiphyllum japonicum* S. et Z.	7
Abies veitchii Lind.	6, 7, 10, 14, 21, 23	*Ceratophyllum submersum* L.	14
Chamaecyparis obtusa Endl.	7, 8, 13, 20, 21	*Corylus heterophylla* Fisch.	5, 14, 18
Chamaecyparis pisifera Endl.	3, 6, 13, 14	*Corylus sieboldiana* Bl.	1, 5, 9
Larix dahurica Turcz. var.	1	*Fagus crenata* Bl.	5, 8
Larix leptolepis Murrey	2, 4, 6, 7, 14	*Fagus japonica* Max.	5, 7
Metasequoia disticha Miki	13	*Hamamelis japonica* S. et Z.	8, 22
Picea bicolor Mayr.	4, 5, 7, 10, 14, 17, 18, 19	*Menyanthes trifoliata* L.	11, 20
Picea glehni Mast.	1, 2, 4	*Prunus maximowiczii* Rupr.	5, 7, 14
Picea koyamai Shiras.	3	*Prunus salicina* Lindl.	14, 18, 19
Picea maximowiczii Regel.	4, 12, 13, 14, 20	*Pterocarya rhoifolia* S. et Z.	5, 7, 17
Picea jezoensis Carr. var.	3, 4, 5, 10, 12, 21, 23, 24	*Pterocarya paliurus* Batal.	13
Pinus parviflora S. et Z.	8, 22	*Quercus crispula* Bl.	4, 7, 8
Taxus cuspidata S. et Z.	4	*Salix* cf. *bakko* Kimura	4
Thuja protojaponica Miki	13, 14	*Sambucus sieboldiana* Bl.	9
Thuja standishi Carr.	7, 8	*Spiraea media* Schmidt.	4
Tsuga diversifolia Mast.	4, 5, 6, 12, 21, 23	*Styrax obassia* S. et Z.	20
Tsuga sieboldii Carr.	3, 7, 8, 10, 15, 21, 22	*Tilia japonica* Simk.	4
		Viburnum furcatum B.	5, 15
Tsuga oblonga Miki	13	*Viburnum opulus* L.	5
Dicotyledoneae		Monocotyledoneae	
Actinidia polygama Miq.	7, 24	*Carex rhinchophysa* C. A. Mey	19
Acer japonicum Thunb.	7, 22	*Iris laevigata* Fish.	11
Acer nikoense Max.	5	*Molinia japonica* Hack.	11
Alnus hirsuta Turcz. var.	7, 8, 18, 19, 23, 24	*Potamogeton gramineus* L.	7
Betula ermani Cham.	12	*Potamogeton perfoliatus* L.	20
Betula platyphylla Sukat.	14, 24	*Sparganium minimum* Fr.	7
Betula grossa S. et Z.	7, 22	*Scirpus* sp.	7, 18, 19

Kantengoya was considered as the Pliocene, and other places as the middle or upper of the Pleistocene because extinct species occurred exceptionally more seldom than that in the *Metasequoia* bed and are unconformable for *Metasequoia* or *Pinus trifolia* bed as in Fig. 2.

These localities with the exception of Kantengoya, Nishinomiya City belong to the Pleistocene, because extinct species are exceptionally few and associated remains are composed of subalpine conifer in Central Honshu, Japan, and these beds are unconformable for the Pliocene bed.

Migration age of Japanese subalpine conifer

The birth-place or the age of development of *Pinus koraiensis* is not clear, but the migration of the subalpine conifer in the Pleistocene of Japan seems to be in the

upper age of Miocene or Palaeocene from the north, because *Pinus koraiensis* and *Picea maximowiczii* occurred in the Pliocene bed at Kantengoya and Japanese alpine plants were considered by Koidzumi (4), to be composed of many endemic species. Judging from the constitution of the Japanese remains, floral change since the Pliocene seems to owe the extinction of many species in Japan. There have been few differentiations of the species since the Pliocene.

The age of differentiation of the Pleistocene species in Japan was considered older than that of upper Miocene and their migration may be sup-
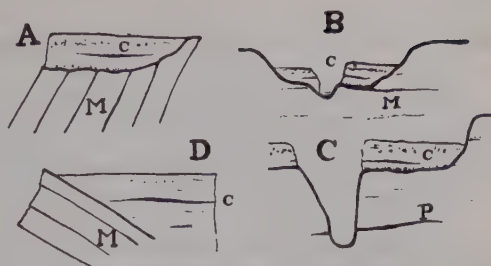

Fig. 2. Diagrammatic profile showing the relation of conifer bed (C) and *Metasequoia* bed (M) or *Pinus trifolia* bed (P).
A Loc. 7: Daimon in Tado, Mie Pref.
B Loc. 8: Conifer bed exposed at river bank of Kodzuhata, Gamo-gun, Shiga Pref.
C Loc. 6: Conifer bed exposed of excavated place of clay in Hara, Turuoka, Gifu Pref.
D Loc. 14: Conifer bed of Manzidani, Nishinomiya City.

posed to be caused by the cold climate at the upper Miocene or Palaeocene. The Pleistocene elements in the Pliocene bed of Japan are considered to have climbed up the mountain, and there are few or no remains of them, as these have a rare chance for preservation. This confirms the belief that most beds of the Pliocene have been more or less related to the sea.

In the Pleistocene they extended down from the mountain, and some migrated from the north. During the intermediate warmer period these species went up the mountain which accounts for the occurrence of many species of broad-leaved evergreen trees at Uegahara in Nishinomiya City, the details of which will be described later in collaboration with Huzita and Kokawa.

The relationship of sea-level to the depositional place

Seed remains of *Ruppia* from various parts of Japan have been excavated from the seven localities of the Pleistocene bed indicating that they had grown at the inlet of a bay. Therefore, the associated bed tells me the height of sea-level at the depositional times and that the average height of sea-level is calculated to have been 42 m in the past.

The range of the depositional height of the *Pinus koraiensis* is wider than that of the *Ruppia*. The higher location of the depositional place was considered as of no relation to sea-level, because the mixture of breccia in the bed and the accompanying water or marshy plants have no relationship to the remains of marine flora or the nearness to the sea. The average height of the *Pinus koraiensis* localities was calculated as 185 m above sea-level. The value of depositional places is adjusted to the sea-level in the past as: 185 m—42 m=143 m.

Depression of the temperature at sea-level of the beds

Determination of past temperature is a troublesome problem (3). I considered the temperature of past times by 10 dominant species belonging to subalpine conifer with the exception of *Alnus hirsuta* and the range of the vertical distribution of the conifer in Central Honshu, Japan, was as is shown in table 3. (The data are from Y. Hayashi (1)).

Therefore, the climate of the growing place in past times is judged as having a lower temperature and more foggy climate than that of the present.

Depression of the temperature of the bed, that is reduced to sea-level was calculated according to the following formula:

Table 3.　Vertical distribution of the dominant species of conifer.

Dominant species	The range of vertical distribution in Central Honshu (m)
Abies homolepis	1000—1700
Abies veitchii	1600—1800
Larix leptolepis	1400—2100
Picea bicolor	1500—2000
Picea maximowiczii	1200—1800
Picea yezoensis var.	1600—2300
Pinus koraiensis	1050—2600
Tsuga diversifolia	1500—2200
Tsuga sieboldii	100—1700
Average height	1217—2033
Average	1625

$$T=[V-(L-S)] \, K$$

T:　Temperature depression.

V:　Average value for vertical distribution of the dominant conifer in Central Honshu, Japan was 1625 m as in Table 3.

L:　Average height of 24 localities of depositional places from Table 1 was calculated to be 185 m.

S:　Average sea-level of the Pleistocene was calculated by the localities of *Ruppia* which indicated it to be above 42 m.

K:　Depression value of temperature is known to be about 0.5° (C) per 100 m upward.

Thus the formula becomes: $7.4° \pm 2.0° \, (C) = [1625-(185—42)] \times \dfrac{0.5}{100}$.

The lower value is confirmed not only by a destitution of broad-leaved evergreen trees, but by a few mixtures of broad-leaved deciduous trees which now grow in the temperate regions of Japan.

Abstract

1) The remains of *Pinus koraiensis* were found in 24 different localities in Japan, associated with about 55 species belonging to 35 genera and 22 families.

2) The following species are new records for the remains in Japan: *Betula ermani, Betula platyphylla, Larix dahurica* var., *Picea glehni, Picea koyamai* and *Pinus parviflora*.

3) Among the associated remains there are 10 dominant species which were found at more than 5 localities. They are growing in foggy places in the mountains of Central Honshu, Japan, about 1500 m above sea-level.

4) The geological age of most localities belongs to the Pleistocene with the exception of one place belonging to the Pliocene. In the middle bed of the Pleistocene some extinct species such as *Tsuga*, *Ceratophyllum* and *Thuja* were found in Manzidani, but they are lacking from the upper Pleistocene as in Egota.

5) The migration of the *Pinus koraiensis* or associated subalpine flora in Japan was considered to be from the upper Miocene and extended down from the mountains during the cold age of the Pleistocene.

6) The depression of temperature at sea-level during the cold age of Pleistocene was calculated roughly as $7.4° \pm 2.0°$ C lower than that of present times. It is confirmed not only by a destitution of broad-leaved evergreen trees, but by a frequent mixture with deciduous broad-leaved trees.

7) *Pinus koraiensis* which is associated with subalpine conifers is considered as an index remain which shows the lowering of temperature in the Pleistocene age in Japan.

Reference

1) Hayashi, Y., Bull. For. Exp. Station Nos. 48, 55, 75 (1951-3) (in Japanese).   2) Huzita, K., Jour. Inst. Poly. Osaka City Univ. Ser. G **2**: 75-88 (1954).   3) Kira, T., Jap. Jour. Ecol. **4**: 45-50 (1954) (in Japanese).   4) Koidzumi, G., Bot. Mag. Tokyo **33**: 193-222 (1919) (in Japanese).   5) Miki, S., Jap. Journ. Bot. **9**: 228-231 (1938).   6) Miki, S., Jap. Journ. Bot. **11**: 377-383 (1941).   7) Miki, S., Minn. and Geol. Japan. No. 9, 106-144 (1948) (in Japanese).   8) Miki, S., Nature and Cult. **1**: 69-116 (1950) (in Japanese).   9) Miki, S., For. Counc. Nagano. 1-17 (1955) (in Japanese).   10) Yamanaka, T., Acta Phytotax. et Geobot. **12**: 192 (1950) (in Japanese).   11) Yabe, H., Journ. Geol. Soc. Jap. **53**: 104 (1947) (in Japanese).

Explanation of the plate (scales mm units)

A. Cone remains of *Picea bicolor* Mayr

    a, from Loc. 5: Uwano in Tonami-gun, Toyama Pref.

    b, from Loc. 19: Peat bed of Ganmanzi, Miyoshi-tyo, Hiroshima Pref.

B. Cone remains of *Picea koyamai* Shiras.

    a, from Loc. 6: Hara in Turuoka, Gifu Pref.

    b, from Loc. 8: Kodzuhata, Gamo-gun, Shiga Pref.

C. Cone remains of *Picea yezoensis* Carr. var.

    a, from Loc. 5: Uwano in Tonami-gun, Toyama Pref.

    b, from Loc. 21: Takadzu in Yama-gun, Shimane Pref.

D. Cone scales of *Abies veitchii* Lind.

    a, from Loc. 6: Hara in Turuoka, Gifu Pref.

b, from Loc. 14: Manzidani, Nishinomiya City.

E.　Twig (a) and cone scales of *Abies homolepis* S. et Z.

a-b, from Loc. 8: Kodzuhata in Gamo-gun, Shiga Pref.

c, from Loc. 22: Kamisohara, Mine City, Yamaguti Pref.

F.　Seed remains of *Pinus koraiensis* S. et Z.

a, from Loc. 22: Kamisohara in Mine City, Yamaguchi Pref.

b, from Loc. 24: Inotuki in Emukai-tyo, Nagasaki Pref.

c, a part of remain of testa, magnified.

G.　Cone remains of *Pinus parviflora* S. et Z. from Loc. 8: Kodzuhata in Gamo-gun, Shiga Pref.

H.　Cone remains of *Larix leptolepis* Murrey

a, from Loc. 7: Daimon in Tado, Mie Pref.

b, from Loc. 6: Hara in Turuoka, Gifu Pref.

I.　Cone (a) and twig (b) remains of *Thuja protojaponica* Miki from Loc. 13: Kantengoya, Nishinomiya City.

J.　Cone remain of *Tsuga diversifolia* Mast. from Loc. 12: Otuti-Pass, Togo-mura, Osaka Pref.

K.　Cone remains of *Tsuga sieboldii* Carr.

a, from Loc. 7: Daimon in Tado, Mie Pref.

b, from Loc. 8: Kodzuhata in Gamo-gun, Shiga Pref.

L.　Fruit remain of *Alnus hirsuta* Turcz. var. from Loc. 23: Clay bed of Kuma-tyo, Ehime Pref.

M.　Scales of *Betula ermani* Cham. from Loc. 12: Otuti-Pass in Togo-mura, Osaka Pref.

a, from outer view,　　b, from inner view.

N.　Fruit and scale remains of *Betula platyphylla* Sukat.

a-b, from Loc. 24: Inotuki in Emukai-tyo, Nagasaki Pref.

c, from Loc. 14: Manzidani in Nishinomiya City.

本　会　記　事

日本学術会議第四期会員候補者
推薦について

　松浦　一氏が推薦を承諾されたので，9 月 28 日本会から同氏候補者推薦届を日本学術会議中央選挙管理会に提出，同日受理された。

支 部 通 信

関東支部

　9 月例会 (9 月 15 日，於東大理植物，講義室)

Dr. St. John (ハワイ大学教授)：ハワイの植物景観.

九州支部

　特別例会 (8 月 31 日，於九大理，第一講義室)
Dr. St. John：ハワイ諸島の景観，植物相と火山.
　第 41 回例会 (10 月 6 日，於福岡女子大) 山本　繁：葉緑体蛋白のアミノ酸定量 (予報). 瀬川宗吉：琉球旅行談.
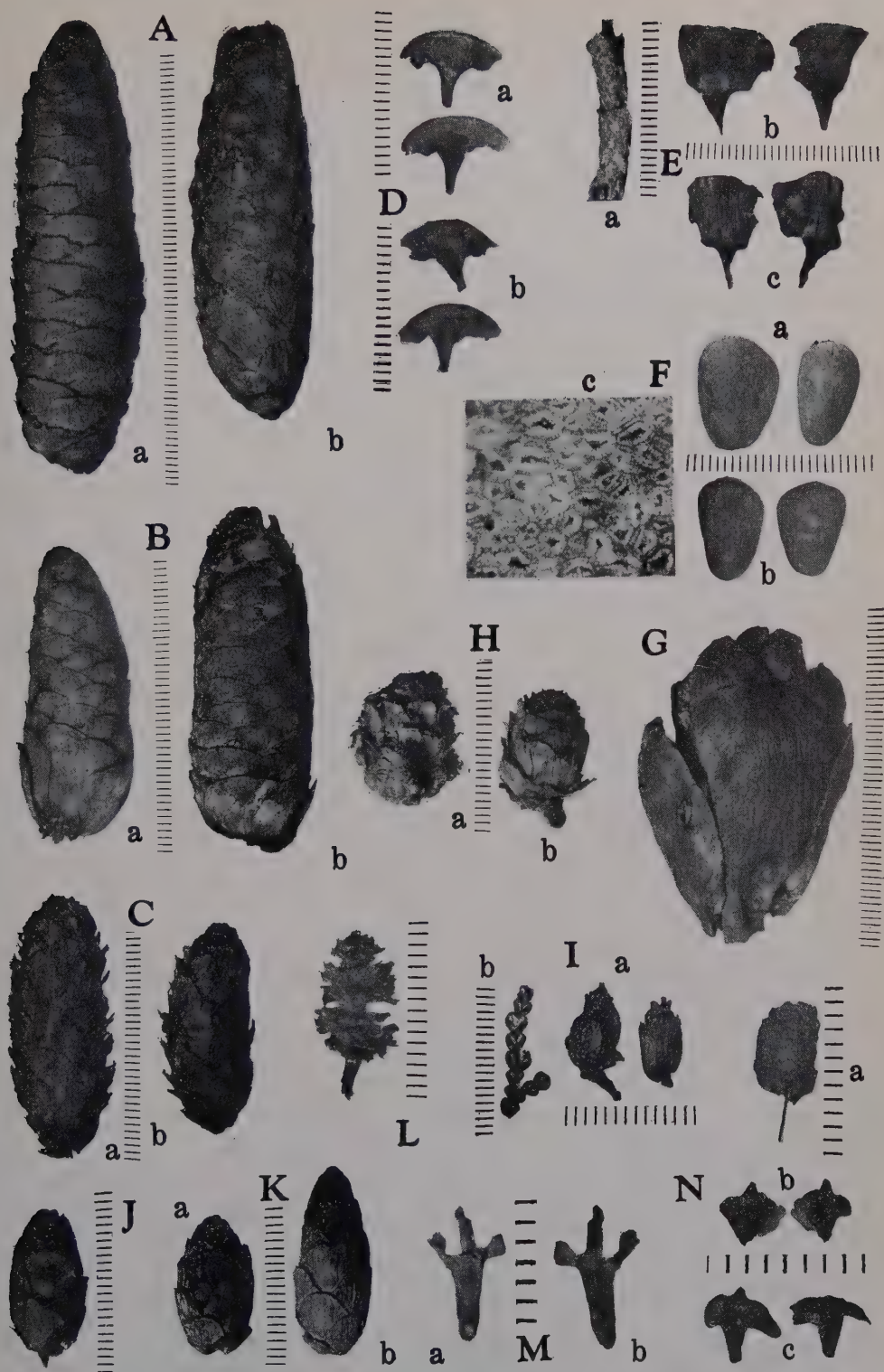
S. Miki : Remains of *Pinus koraiensis* S. et Z. and associated remains in Japan.

Morphology and Development of the Sinker in *Pecteilis radiata* (Orchidac.)

by Masao Kumazawa*

熊沢正夫*： サギソウの地下器官の形態と発生

Ogura (1953) studied the comparative morphology and anatomy of the sinkers of 27 species belonging to 10 genera of the Ophrydinae, and discussed the morphological nature of the organ. In his paper, the vascular behaviours within the full-grown sinker were examined. However, the sinker development, most significant in understanding the morphological nature of the organ, was not studied in any species.

The sinker development of the Ophrydinae was studied first by Irmisch (1850) in *Platanthera bifolia,* later by Nothdurft (1955) in a species of *Serapias.* Between the two, there were some descriptions, among which Sharman's (1939) on *Orchis mascula* entered in details. In the present paper the sinker of *Pecteilis* (*Habenaria*) *radiata* Rafin. is described which somewhat differs from that of *Orchis mascula* in respects of morphology and development.

Material and methods

The plant grows wild in the vicinity of Nagoya and materials were obtained there in the growing season, but cultivated plants were also used. Hundreds of plants were examined during the years from 1949 to 1955. The anatomical studies were made usually from microtome serial sections, stained with safranin and fast green or with safranin and Delafield's haematoxylin.

External morphology

Fig. 1 shows the plant whose leaf tips have just emerged on the earth surface at the beginning of April. Several leaves (f_1, f_2, f_3) are found on the short axis produced from the top of the brown tuber densely covered with the root hairs. At the node of f_1, three absorbing roots are elongating horizontally. A decayed thin scale (s_3), brown in colour, embraces the base of the erect stem, concealing a small bud (s_3b), 1 mm in length, in its axil. Fig. 2 shows the subterranean parts of a plant in the middle of June. In the fertile plant, the erect stem is now somewhat elongated, and several absorbing roots, arising at the nodes of f_1 and f_2, are longer and brown in colour, covered with root hairs. No lateral rootlets are produced. Besides the absorbing roots, one root-like organ, (f_2b) and soon later one more (f_1b)

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* Biological Laboratory, Department of General Education, Nagoya University, Nagoya, Japan.
名古屋大学教養部生物学教室

appear respectively from the parts[1] near the axils of f_2 and of f_1. In external view these organs, in this phase of development, can only be distinguishable from the absorbing root by their white colour and their lack of root hairs. The root-like organ, the young sinker continues the horizontal elongation until the flowering season (August), often exceeding lastly 10 cm in length. Then the tip turns downwards, coloured in brown (Figs. 4 and 5), and gradually increases its length and diameter, being covered with hairs (Fig. 6). Until the end of September, the brown part develops into a spherical tuber, 1 cm or less in diameter (Fig. 7). The tuber has a neck (Fig. 7, n), through which it is connected with the horizontal slender part (stalk) of the sinker. In November the aerial parts begin to die and all the subterranean organs, except the new tubers which remain alone through the winter, shrink and fall into decay sooner or later. Non-flowering plants also follow the same processes.
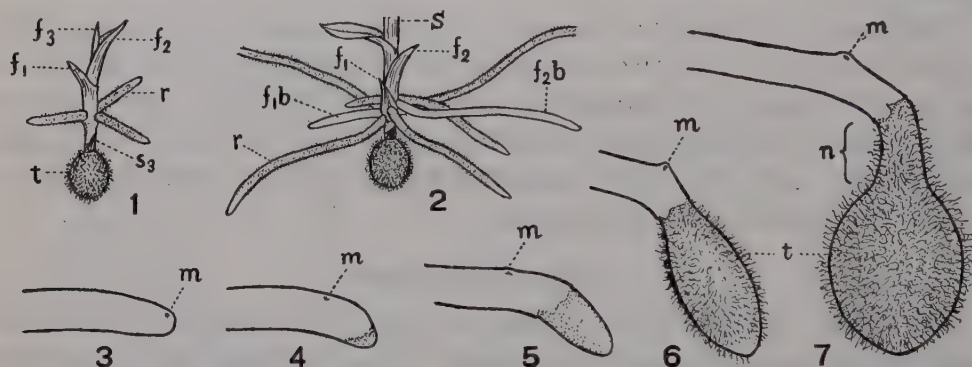
Fig. 1. A plant at the beginning of April. Fig. 2, A plant in early summer, the erect stem not shown. Figs. 3-7, Distal parts of the sinker, showing the successive development of the tuber. f_1, f_2, f_3, leaves on the erect stem. s_3, decayed scale leaf. f_1b, f_2b, elongating young sinkers, S, erect stem. r, absorbing roots. t, tuber. m, opening. n, neck.

The plant just germinated from the seed is represented by a spherical protocorm as is usual with the Orchidaceae. Within a few months several foliar leaves are produced at the upper pole of the protocorm which increases its length and diameter gradually and develops into the first tuber covered with root hairs. The tuber has neither an apical meristem nor a root cap. The first absorbing root arises at the second node, and the first sinker primordium usually at the axil of the third or fourth leaf. The tuber derived directly from the protocorm dies without producing the flowering axis in the first year of germination.

Structure of the mature tuber

The main part of the full-grown tuber observed in Autumn (Figs. 7 and 13) is a typical tuberous root having a rudimental rootcap-like tissue (Fig. 13, c), starch

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1) More accurately speaking, the parts slightly deviate laterally from the real axil or above it.

reserving fundamental tissue and monostele consisting of polyarch radial bundles. The neck encloses a terminal bud covered with a thin scale leaf (Fig. 13, s_3) in its central cavity, which communicates with the outside through a small opening (m). The opening, located in a place 5–10 often 20 mm from the top of the neck, is so small that it is hardly visible to the naked eye, and its existence was not described by Ogura (1953); but sometimes marginal part of the opening protrudes slightly, thus the position of the opening is easily suggested. As shown by the semidia-grammatic transection through the neck (Fig. 12), the leaves f_1, f_2 and s_3 within the neck have the axillary buds f_1b, f_2b and s_3b respectively. These leaves and buds correspond to the respective organs indicated by the same symbols in Figs. 1 and 2. The scale leaf s_3 is a few cell layers in thickness, no vascular tissue being
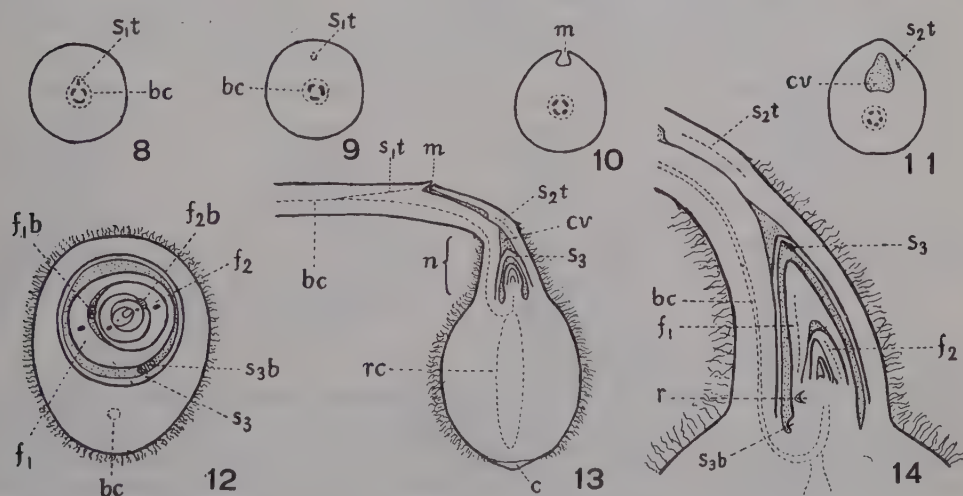
Figs. 8–11. Successive transections through the distal part of the stalk shown in Fig. 13. Fig. 12. Transection through the neck. Fig. 13. Longisection of the distal part of the sinker. Fig. 14. Longisection of the neck. bc, stele of the sinker axis. s_1t, s_2t, trace bundles belonging respectively to the first and the second leaves on the sinker axis. cv, cavity communicating with the outside through the opening m. s_3b, axillary bud of the scale leaf s_3. rc, stele of tuber. c, root pocket. Other abbreviations as in the foregoing figures.

observed except only in one example among hundreds of tubers studied. f_1b and f_2b are the primordia of the sinkers which elongate in the following growing season, while s_3b remains undeveloped throughout and shares the fate of the mother tuber, dying usually in late autumn of the following year.

On one side of the neck, there is a tubular stele consisting of several collateral bundles (Figs. 12 and 14, bc). This is the stele of the sinker or stalk and bends at the base of the terminal bud and goes into it. The stele of the tuber (Fig. 13, rc) is separated from the curved part of the stalk stele. Figs. 8–11 show the transections through the distal part of the stalk shown in Fig. 13. s_1t is a rudimental collateral bundle which is separated from the upper side of the stalk stele and comes to an end (Fig. 13, s_1t) near the opening. This is a leaf trace belonging to the first leaf

on the sinker axis. A rudimental vascular strand s_2t (Figs. 11 and 13), observed in about half the plants studied by the writer, is regarded also as a leaf trace belonging to the second leaf, although it fades out at both ends.

In the older tuber, the rootcap-like tissue (root pocket) peels off completely and the tuber apex is also covered with the rhizoderm having root hairs.

Development of the sinker

In autumn, the axillary buds s_3b, f_1b and f_2b within the neck are initiated exogenously in an acropetal order, each as a cup-shaped process. The process develops to be 1 mm in length and then its development is suppressed until the following year. The bud in this stage (Fig. 15) has no peculiar features, the shoot apex being enclosed by the primordial leaves s_1 and s_2. In the coming late spring, f_2b begins first to exhibit the elongating growth as a sinker by means of the rib meristem (Fig. 16, rm), which appears at the zone behind the node of s_1. However, the primordial leaves s_1 and s_2 scarcely grow further, consisting of a small number of immature cells. Two procambial strands, the one detached from the upper side of the sinker stele (Fig. 17, s_1t), the other from the lower side (Fig. 17,
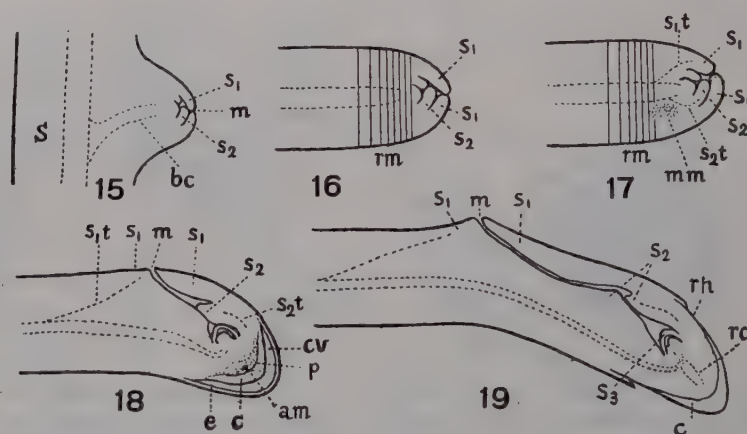
Figs. 15-19. Longisections through the distal part of sinker, showing the successive stages of development. s_1, s_2, s_3, the first to the third leaves of sinker. rm, rib meristem. mm, mass meristem. am, apical meristem. e, epidermis. cv, slit formed between the epidermis and underlying tissue. p, protoderm. rh, rhizoderm. c, root pocket. Other abbreviations as in the foregoing figures.

s_2t), are differentiated acropetally and the one goes into s_1, the other into s_2. Therefore, s_1 is undoubtedly an inverted prophyll and s_1t is its leaf trace. s_1 and s_2 never produce their axillary buds even if in their last stages of development.

In a later stage of sinker elongation, a mass meristem (Fig. 17, mm) becomes differentiated from some periblem cells near the base of s_2t. The meristem consists of rather small cells rich in protoplasm; however, its outer boundary layer is not clearly distinguishable from the surrounding tissue. After the flowering season is over and the sinker is almost fully elongated, the distal lower halves of the horizontal sinker itself and of the primordial leaves exceed considerably the opposite halves in cell elongation. As the results the upward bending of the sinker axis

takes place (Fig. 18); s_1 and s_2 are prolonged on one side more remarkably than on the other side; the small procambial strand (Fig. 18, s_1t) is dissected from the sinker stele and the opening (m) is displaced towards less distal part of the sinker. Meanwhile, the cells between the epidermis and the meristem become vacuolated and a slit (Fig. 18, cv) appears between the epidermal and subepidermal layers. The protoderm and probably the calyptrogen are established from the outermost part of the meristem. The vacuolated cell group (Fig. 13, c) mentioned above covers the apical meristem firmly as if it were a root cap. Therefore, it is in reality a sort of root pocket, as the calyptrogen produces only a few cell layers. After the epidermis is destroyed a typical root covered with the root pocket becomes exposed as if it were a direct continuation of the sinker axis (Fig. 19). The part of the root structure is rapidly enlarged, being covered with the rhizoderm having root hairs on its surface, thus the root tuber is formed. It must be noticed that the differentiation of rhizoderm proceeds across the part of the root stucture even to the subepidermal pa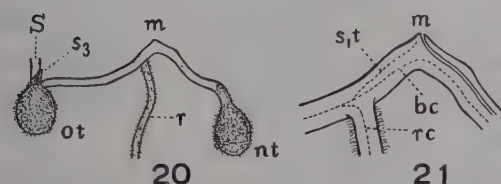rt of the neck. This results in the peculiar features of the neck: although the neck itself is not a root as its ontogeny shows, its surface layer is represented by the rhizoderm having root hairs after the epidermis is peeled off.
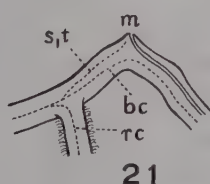
Fig. 20. Abnormal sinker with an absorbing root r, arising at the axil of s_3. Fig. 21. A part of the foregoing figure, showing the vascular behaviours. ot, mother tuber. nt, new tuber. Other abbreviations as in the foregoing figures.

When the sinkers, f_1b and f_2b, are artificially injured in the early stage of their development, s_3b often develops into a sinker. Such compensatory development of the sinker has been described in other species of the Ophrydinae by Stojanow (1917), Ziegenspeck (1930), Sharman (1936) and others. Although the normal sinker produces no absorbing roots, the writer has often found the compensatory sinker, on which a single absorbing root arises at the base of the prophyllar trace s_1t (Figs. 20 and 21). The development of such an absorbing root may be interpreted as the compensatory response to the injury of the normal absorbing roots rather than as a phenomenon confined to the compensatory sinker alone.
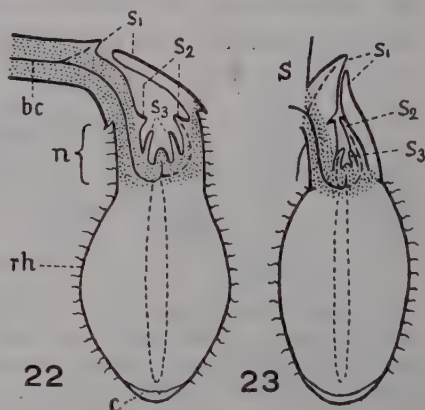
Discussion

It has been pointed out by some authors that the sinker of the Ophrydinae is nothing but a normal axillary bud in the early stage of its development. This holds true also in the case of *Pecteilis*. The sinker of *Pecteilis* is, however, elongated extraordinarily by means of the rib meristem situated immediately behind the first node; and then an adventitious root originates from the node of the second primordial leaf and becomes tuberous. A mass meristem, from which the main part

of the root is differentiated, is initiated in the periblem near the shoot apex, so it is of mesogenous origin rather than of exogenous, according to the terminology of Guttenberg (1940). Although the roots of the Ophrydinae were often described as of exogenous origin by some authors such as Stojanow (1917), Fuchs and Ziegenspeck (1925), and Burgeff (1932), they may also be of mesogenous, i. e. of the outer periblem origin.

The morphological nature of the sinker in the Ophrydinae has variously been interpreted (vid. Ogura, 1953). Ogura considered that the distal part of a sinker is a combined system of some roots, and the proximal part a combined system of cauline and radical characters. Thus he applied the term rhizophore to the latter part of the sinker. So far as the present study of *Pecteilis* concerns, it is quite certain that the proximal slender part (stalk) of the sinker is the hypopodium of an axillary shoot and of typical cauline nature, the distal tuberous part a single adventitious root originating from the second node. The morphological peculiarities are observed only in the part between the stalk and the tuber, i. e. in the neck. The ontogeny shows that the neck corresponds mainly to the parts between the first and the third nodes of a lateral shoot. Accompanied by the development of the adventitious root, the shoot apex itself falls into a cavity formed by the characteristic growth of those parts mentioned above. The rhizoderm differentiation which is usually confined to the root itself, proceeds to the subepidermal part of the neck across the part of root structure. The neck, accordingly, loses its epidermal layer and is covered with the rhizoderm having root hairs. Then we may call the neck a sort of the periclinal chimera consisting of the stem and root. If any special term is prepared, it should be applied to the neck alone. The cases, in which

Figs. 22 and 23. Diagrams showing the construction of full-grown sinkers. Fig. 22. *Pecteilis radiata*. The slender proximal part of the sinker not shown. Fig. 23. *Orchis rupestris*, the usual tuberous type. The stippled part corresponds by origin to the cauline axis of the lateral shoot. c, root pocket peeling off in the later stage of tuber. Other abbreviations as in the foregoing figures.

an organ other than the root is covered with rhizoderm, have been described (e. g., the cotyledonary petiole of some geophilous species in the Ranunculaceae, Berberidaceae and Umbelliferae—Haccius, 1953).

All the sinkers of fusiform, handform and tuberous types observed in many species of the Ophrydinae show one and the same fundamental construction derived from an axillary shoot accompanied by an adventitious root (Fig. 23). This holds true also in the case of the sinker of *Pecteilis*. Most of other species, however, differ from *Pecteilis* in the following respects: 1) the adventitious root is initiated

and grows at a very early stage of sinker development; 2) the hypopodium or the sinker does not elongate very long; 3) the rhizoderm differentiation is confined to the part of typical root nature.

The development of the sinker, which was treated by Ogura as of the stoloniferous type, will be described in the following paper.

Summary

In the early stage of development, the sinkers of *Pecteilis radiata* are typical lateral buds arising at the axils of the fourth and fifth leaves on one monopodium. In summer the hypopodium of the lateral bud is elongated extraordinarily by a rib meristem and is hardly distinguishable from the absorbing roots except by its white colour and the absence of root hairs. Then a mass meristem appears within the periblem near the second node, and later it develops into a tuber.

The full-grown sinker consists of three parts—the stalk, the neck and the tuber. The stalk corresponds to the hypopodium of a lateral shoot and is a typical cauline axis; the tuber, the distal part of the sinker, is a single adventitious root of the mesogenous origin. The calyptrogen is not so active that a root pocket derived from the periblem of the mother axis covers the tuber apex firmly as if it were a real root cap, but in the older tuber the root pocket is peeled off completely and the tuber apex is also covered with the rhizoderm having root hairs. The neck is represented by the connecting part between the stalk and the tuber, and morphological peculiarities are observed in this part alone. The neck has a central cavity, at the bottom of which the terminal bud of the lateral shoot is situated. The ontogeny shows that the neck is derived from the parts between the first and the third nodes of a lateral shoot by the characteristic growth of the parts. The rhizoderm is, however, differentiated not only at the part of typical root nature but also at the part of the neck, and then the epidermis of the neck is replaced by the rhizoderm after it is peeled off. Therefore, the neck may be said to be a sort of the periclinal chimera consisting of the stem and root.

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Über das Anthocyanin in den dunkelpurpurnen Blüten von *Tulipa Gesneriana* L.*

Studien über die Physiologie von Liliaceen I

von Mannen SHIBATA**

柴田萬年**: 暗紫色花チューリップのアントシアニンについて*

（百合科植物の生理学的研究 I）

Der Blütenfarbstoff von *Tulipa Gesneriana* L., dem verbreiteten und beliebten Gartenpflanzen aus der Familie Liliaceen, bildet den Gegenstand vorliegender Mitteilung. Diese Gartenpflanze wird in der Umgebung von Stadt Tonami in Toyama Präfektur in grossem Umfang gepflanzt und blüht in der Frühlingszeit fast auf einmal auf.

Anfang Mai 1953 habe ich eine Gelegenheit gehabt, eine Menge Blüten der genannten Pflanzen abzupflücken und in frischem Zustand zu chemischen Untersuchungen heranzuziehen.

Als Untersuchungsmaterial benutzte ich eine leicht in Menge anschaffbare, dunkelpurpurn*** blühende Sorte „ Queen of night ", die im Jahre 1950 von den Niederlanden eingeführt wurde.

Das Ausziehen des Anthocyanins wurde wie üblich durch Einlegen des Materials in 1-proz. methanolische Salzsäure ausgeführt, und aus dem dunkelroten Extrakt wurde der Farbstoff bequem als Bleiverbindung niedergeschlagen. Nach der Behandlung mittels chlorwasserstoffhaltigen Methanols wurde der Farbstoff mit viel Äther ausgefällt. Aus der gefällten Farbstoffraktion, die noch mit kleiner Menge Begleitstoffen verunreinigt ist, kann man das Anthocyanin ohne Schwierigkeit dadurch reinigen, dass man die obige flockige Fällung in 1-proz. kalte äthanolische Salzsäure aufnimmt und mit viel Äther nochmals ausgefällt.

Der ausgefällte amorphe Farbstoff wird in wenigen kaltem Äthanol aufgenommen, filtriert und sofort in 1/2 Vol. kalte 20–proz. äthanolische Salzsäure eingetragen.

Schon nach kurzem Stehen beginnt die Kristallisation des Anthocyaninchlorids. Das abgesaugte Kristallisat war wie erwartet gering, die Ausbeute betrug 0.14 Proz. des frischen Ausgangsmaterials.

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* Vorgetragen an der jährlichen Versammlung der Botanischen Gesellschaft von Japan, die den 13. Okt. 1955 in Hiroshima abgehalten wurde.

** Biologisches Institut der Universität zu Toyama, Japan. 富山大学文理学部生物学教室

*** Sie wird gewöhnlich von Blumenzüchtern „ schwarz " genannt.

Vor ungefähr zwanzig Jahren haben G. M. Robinson und R. Robinson mit Hilfe einer von ihnen ausgearbeiteten qualitativen Methode den Blütenfarbstoff mehrerer Sorten von *Tulipa Gesneriana* L. untersucht und berichtet, dass er sich meistens als Glukosid des Pelargonidins oder in einigen Sorten überdies mit einer kleinen Menge von Cyanidinglukosid gemischt befinde.

Nach dem Ergebnis meiner Versuche, die die Reindarstellung des Farbstoffs zum Ziel hatten, wurde der Befund Robinsons als verschieden erkannt, obwohl seine Sorte von *Tulipa* nicht mit meiniger identisch war. Nach meinen quantitativen Versuchen gab 1 Mol Anthocyaninchlorid 1 Mol Glukose, 1 Mol Rhamnose und 1 Mol zuckerfreien Farbstoff und der letztere konnte chemisch, papierchromatographisch sowie auch spektrophotometrisch mit Delphinidinchlorid identifiziert werden.

Die Verteilungszahl dieses Farbstoffs war ca. 7.2.

Aus dem oben gesagten liess sich das Anthocyanin als ein Delphinidinrhamnoglukosid erkennen. Daher wird der neue Blütenfarbstoff provisorisch „Tulipanin" genannt. Wegen der Knappheit an Menge ist es aber mir leider bis jetzt noch nicht gelungen, die Lage der Zuckerbindung festzustellen.

Beschreibung der Versuche

Isolierung von Tulipaninchlorid.

17.35 kg frische, von Blütenständen abgenommene Kronenblätter* („ Queen of night") wurden mit 18 l 1-proz. methanolischer Salzsäure übergegossen, 9 Stunden lang stehen gelassen und hierauf von dem roten Extrakt durch Pressen abgetrennt. Der Extraktrückstand wurde von neuem in 8 l Methanol über Nacht liegen gelassen, abgenutscht und mit 2 l Methanol gut gewaschen. Die vereinigte Extraktionsflüssigkeit ist zähflüssig, da sie noch viel schleimige Begleitstoffe enthählt. Aus diesem Filtrat fällt man durch Zusatz von gesättigter Bleiacetatlösung den Farbstoff in rohem Zustand als wattenartiges, blaues Bleisalz aus, wobei die methanolische Mutterlauge noch etwas schmutzig purpurn gefärbt erscheint.

Dieses Bleisalz, welches sofort abgenutscht und nacheinander mit Wasser und Äthanol gründich gewaschen wurde, wiegt in lufttrockenem Zustand ca. 708 g. Es ist klar, dass mit diesem blauen Bleisalz eine beträchtliche Menge von Bleichlorid mitgerissen wird. Dieses Bleisalz wird in einer Reibschale fein pulverisiert und in 1500 ccm 5-proz. methanolischer Salzsäure unter Wasserkühlung und Anrühren mit Glasstab in mehreren Portionen eingetragen, wobei der Farbstoff sehr leicht als Chlorid in Lösung geht.

Nach dem Abfiltrieren des ausgefällten Bleichlorids, das weiter mit 800 ccm Methanol gut ausgewaschen wird, versetzt man das vereinigte tiefrote Filtrat mit fünffachem Volumen Äther, wobei das Anthocyaninchlorid als flockiger Niederschlag

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* Dieses Material wurde durch Gütigkeit von Herrn M. Neo in seinem Garten gesammelt, wofür ich ihm auch an dieser Stelle herzlich danken möchte.

ausfällt, welcher sich alsbald zu einer dichteren Masse am Boden des Kolbens zu-
sammenzieht.　Nach dem Abdekantieren der oben stehenden Mutterlauge löst man
nun die Farbstoffmasse in 1-proz. äthanolischer Salzäure auf, filtriert und fällt den
Farbstoff durch Zugabe von viel Äther aus.　Der erhaltene amorphe Niederschlag
wird nach übernachtigem Stehen abgenutscht und sofort in kleine Menge von kaltem
Äthanol eingetragen, wobei er allmählich in Lösung geht.　Die dunkelrote Äthanol-
lösung des Anthocyanins wird filtriert und beim Znsatz von 1/2 Volumen 20-proz.
äthanolischer Salzsäure tritt das Tulipaninchlorid als tief schokoladenfarbige, fast
schwarz aussehende Masse teilweise auf.　Nach dem Stehen über Nacht im Eisschrank
ist die Auskristallisation vollständig.　Die abgesaugten Kristalle bestanden aus fast
schwarz aussehenden prismatischen Nadeln, die ca. 22 g wogen.　Die Mutterlauge
wird zu weiterer Ausbeute unter Zugabe von weniger 20-proz. äthanolischer Salzsäure
noch einige Tage stehen gelassen.　Diese nachherigen Ausscheidungen sind nur gering
(ca. 3 g) und etwas unrein.　Die Ausbeute betrug nämlich 0.14 Proz. des frischen
Ausgangsmaterials.

　　　Dieser rohe Farbsfoff wird in einer möglichst kleiner Quantität heissen Wassers
gelöst und filtriert und das Filtrat wird mit gleichem Volumen 20-proz. äthanolischer
Salzsäure vermischt.　Schon nach kurzem Stehen tritt eine Trübung der Lösung ein
und beginnt die Kristallisation des Tulipaninchlorids, was gewöhnlich in einigen
Stunden endet.　Die Verbindung bildet dabei äusserst feine, schokoladenfarbige,
prismatische Nädelchen.　Drei- bis viermalige Wiederholung dieses Verfahrens brachte
das Tulipaninchlorid in vollkommen reinen Zustand, wobei man unterm Mikroskop
schöne rotbraune Nädelchen sehen konnte, welche einen prachtvollen Goldglanz gaben.

　　　Beschreibung des Tulipaninchlorids.

　　　Tulipaninchlorid kristallisiert, wie aus Fig. 1 ersichtlich, zu prismatischen Nä-
delchen, die sich beim Erhitzen in einem Schmelzpunktröhrchen von 177°–178° (un-
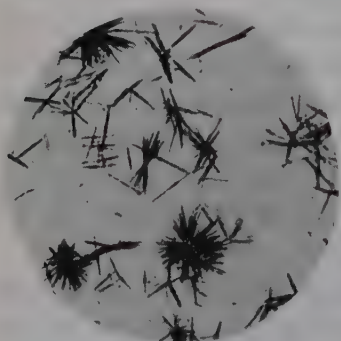korr.) ab zusammenziehen und zersetzen.

Fig. 1.

Hinsichtlich der Verteilung zwischen *iso*-Amyl-
alkohol und 0.5-proz. Salzsäure verhielt sich das Tu-
lipaninchlorid monoglykosidisch, wie es bei den
Rhamnose enthaltenden Diglukosiden gewöhnlich der
Fall ist.　Nämlich geht ca. 7.2 Proz. des Tulipanin-
chlorids aus wässriger Schicht in den *iso*-Amylalko-
hol über.　Wie es sich aus den Details unten ersehen
lässt, ist dieser *Tulipa*-Farbstoff in manchen Eigen-
schaften dem R. Robinson u. A. (1934) aus den blauen
Blüten von *Salvia patens*, dem von C. Kuroda (1935)

aus dem himmelblauen Blüten von *Commelina communis* var. *hortensis* isolierten
Delphin und dem von K. Hayashi (1936) aus der azurblau blühenden Sorte von
Hyacinthus orientalis L. isolierten Hyacin ähnlich.

Um den Vergleich zu erleichtern, werden einige leicht ermittelbare Eigenschaften der drei Verbindungen in folgender Tabelle zusammengestellt:

	Delphinchlorid*	Hyacinchlorid*	Tulipaninchlorid
Kristall-form	kurze, flache blattartige Kristalle	linsenförmige Täfelchen	prismatische Nädelchen
Zersetz-ungspunkt		188° (von 185° ab zusammenziehen)	177°–178° (unkorr.)
Löslichkeit	in kaltem Wasser, Alkohol u. verd. Salzsäure sehr schwer löslich (od.unlöslich)	in kaltem Wasser sehr schwer, in heissem Wasser löslich; in kaltem Äthanol sehr schwer löslich; in verd. HCl verhält es sich fast gleich wie Wasser	in kaltem Wasser, Äthanol und verd. Salzsäure ziemlich löslich
Bildung von Pseudobase	beim Erhitzen in H_2O od. Äthanol tritt die Pseudo-basebildung leicht ein	dieselbe Reaktion tritt nur schwer ein	beim Erhitzen in Wasser od. Äthanol tritt die Pseudobase-bildung sehr schwer ein
Reaktion mit Soda-lösung	rein blau und bald grün	rein blau, nach einigen Sekunden blaugrün und bald grün	rein blau, nach einigen Sekunden gelbbraun
Reaktion mit Nat-ronlauge	dieselbe Farbänderung, aber geht die Reaktion sehr schnell vor sich	momentan blau, dann plötzlich grün u. bald gelbbraun	momentan rein blau, dann grün und bald gelbbraun
Reaktion mit Na-Acetat	violett-blau	violett	violett, ziemlich stabil
Reaktion mit $FeCl_3$	blauviolett (in Wasser) violettblau (in Äthanol)	tief blau, aber mit einem Stich ins violett (in Wasser); tief himmelblau (in Äthanol)	violett (in Wasser); blauviolett (in Äthanol)
Reaktion mit Blei-acetat	königblauer Niederschlag (royal blue)	blauer Niederschlag	tief blauer Niederschlag
Reaktion mit Pik-rinsäure	kein Niederschlag	kein Niederschlag	kein Niederschlag

Die Analysenzahlen des Tulipaninchlorids sind wie folgt:

3.448 mg lufttrockene Subst. verloren im Vakuum (2 mm Hg) bei 105° über P_2O_5 0.420 mg an Gewicht.

3.028 mg wasserfreie Subst.: 5.424 mg CO_2; 1.168 mg H_2O

$C_{27}H_{31}O_{16}Cl$ Ber. C 50.10, H 4.83
                              Gef. C 48.88, H 4.32

$C_{27}H_{31}O_{16}Cl·5H_2O$ Ber. H_2O 12.22
                              Gef. H_2O 12.18

* Zitiert nach K. Hayashi: Acta Phytochim. **9**: 28 (1936)

Hydrolytische Spaltung von Tulipaninchlorid.

Zur sauren Hydrolyse wird 1.3017 g reinen Tulipaninchlorids ($C_{27}H_{31}O_{16}Cl \cdot 5H_2O$) in 100 ccm warmem Wasser gelöst, 60 ccm konz. Salzsäure (sp. Gew. 1.18) gefügt, 3 Minuten lang gekocht, und nachher über Nacht in Eiswasser gestellt. Beim Abkühlen mit Eiswasser schied sich der zuckerfreie Farbstoff in den fächer- oder fischschwanzförmigen Tafeln fast qauntitative aus (Fig. 2). Das abgespaltene Aglykon
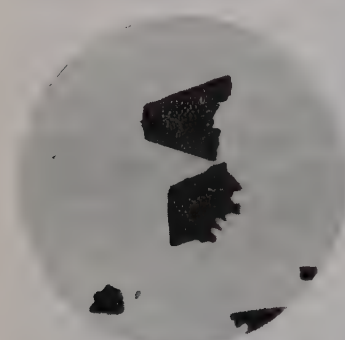

Fig.2.

(Delphinidin) fiel dabei fast gänzlich aus und konnte abgenutscht werden. Die Kristalle sehen in der Masse fast schwarz aus und zeigten einen prachtvoll grünen Glanz. Sein Gewicht beträgt in exsikkator-trockenem Zustand (über $CaCl_2$) 0.6118 g, nämlich ca. 47 Proz. des Tulipaninchlorids. Es besitzt die charakteristische Lösungsfarbe, das Absorptionsspektrum und die sonstigen Eigenschaften des von Willstätter beschriebenen Delphinidinchlorids.

Die saure wässrige Lösung ist rosa gefärbt, und enthält dann nur Spuren von Delphinidin. Man wäscht nun die saure Lösung und den abgesaugten Farbstoff mehrmals mit Äther aus, und die ätherischen Auszüge hinterlassen nach dem Verdampfen keinen merklichen Rückstand, woraus zu schliessen ist, dass etwaige bei gewissen Anthocyanen gebunden angetroffene organische Säure hierbei nicht vorhanden ist.

Mit Äther erschöpfend ausgezogene, saure Flüssigkeit, welche die abgespaltenen Zucker enthält, wurde zur Entfärbung mit *iso*-Amylalkohol gründlich geschüttelt. Die Amylalkoholreste wurden durch Ausschütteln mit Äther entfernt, dann mit Sodalösung neutralisiert, und die farblose Lösung wurde unter vermindertem Druck zum Trocken verdampft. Aus dem hinterbleibenden Rückstand wird weiter in üblicher Weise das Osazon dargestellt. Das gebildete Osazon wird mit Aceton behandelt, aus dem in Aceton löslichen Teil gewinnt man ganz einheitliche Nadelbüschel, welche nach einmaligem Umlösen den Schmp. 180° zeigten, und aus dem in Aceton unlöslichen Teil gewinnt man gelbe Nadeln vom Schmp. 204°. Wird das erstere mit reinem Rhamnosazon vom Schmp. 183° und das letztere mit reinem Glukosazon vom Schmp. 204-5° gemischt, so bemerkt man keine Schmelzpunktserniedrigung, woraus zu schliessen ist, dass die Zucker Rhamnose und Glukose sind. Zugleich ist mit Hife von papierchromatographischer Methode Glukose und Rhamnose bestätigt.

Beschreibung des Aglykons (Delphinidinchlorid)

Der zuckerfreie Farbstoff von *Tulipa*-Blüten verhält sich in allen Punkten gleich wie Delphinidin, das zum erstenmal von R. Willstätter u. A. gewonnene Anthocyanidin aus *Delphinium*-Blüten.

Aus 99 proz. Äthanol unter Zusatz von ein wenig 20 proz. äthanolischer Salzsäure umkristallisiert, fällt das Delphinidin meistens als Aggregat von äusserst

feinen Nädelchen (Fig. 3). Die qualitativen Reaktionen des Farbstffs mit NaOH, Na_2CO_3, $FeCl_3$ u. a. und dessen Reduktionsvermögen der Fehlingschen Lösung in der Kälte usw. stimmt mit der Literaturangabe gut überein. Das Aglykon enthält keine Methoxylgruppe nach der Methode von Zeisel-Pregl.

Das im Chlorcalcium-Exsikkator getrocknete und unter vermindertem Druck bei 105° getrocknete wasserfreie Delphinidinchlorid (ohne besondere Reinigung) ergibt die folgende Analysenzahlen.

2.800 mg lufttrockene Subst. verloren im Vakuum (2mm Hg) bei 105° über P_2O_5 0.185 mg an Gewicht.

2.619 mg wasserfreie Subst.: 5.058 mg CO_2; 0.714 mg H_2O

$C_{15}H_{11}O_7Cl$ Ber. C 53.15, H 3.25
                            Gef. C 52.78, H 3.06
$C_{15}H_{11}O_7Cl \cdot 1\frac{1}{2} H_2O$ Ber. H_2O 7.38
                            Gef. H_2O 6.61

Die Absorptionsspektren von *Tulipa*-Farbstoffen.

Die Absorptionsspektren von dem in der Natur auftretenden Anthocyanidin, wie K. Hayashi in seiner dritten Mitteilung über die spektrographischen Untersuchungen der Farbstoffe von Anthocyanidintypus ausführlich beschrieben hat, wurden zur Kon-
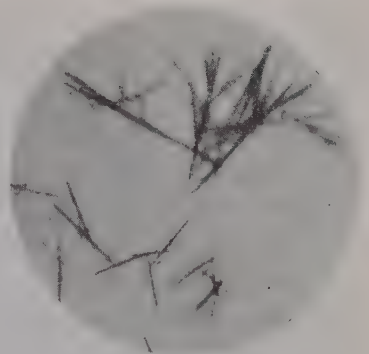
Fig. 3.
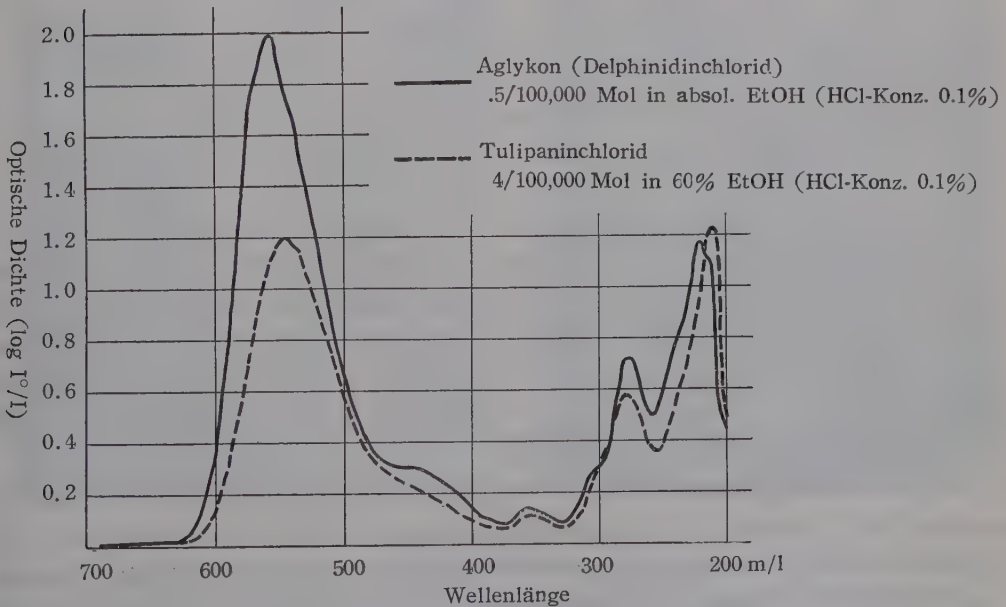
Fig. 4.

stitutionsermittelung des hier aus *Tulipa*-Blüten isolierten, zuckerfreien Farbstoffs nutzbar gemacht. Hierbei werden auch die Absorptionskurven des Glukosids sowie des Aglykons dargestellt. Zum Spektrographieren wurde das Aglykon in absolutem Äthanol

$(5\times10^{-5}$ Mol) und das Glukosid in 60 proz. Äthanol $(4\times10^{-5}$ Mol) gelöst, wobei der Chlorwasserstoffgehalt der Lösungsmittel zu 0.1 Proz. eingestellt wurde (Fig. 4).

Aus der Kurvenform und Absorption in der Nähe von 358 mμ, wie in Fig. 4 angegeben ist, kann man darauf schliessen, dass das *Tulipa*-Aglykon nichts anderes als Delphinidin selbst ist. Noch weiter stimmt *Tulipa*-Aglykon mit Delphinidin-Präparat von K. Hayashi nach papierchromatographischer Methode gut überein.

Zum Schluss möchte ich Herrn Prof. Dr. K. Hayashi in der Tokyo Universität für Erziehung für seine vielseitige Belehrung und stetige Anregung meinen tief gefühlten Dank aussprechen.

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A Large Silicified Wood of *Aleurites* from the Miocene of Isikawa Prefecture, Honsyu[*]

by Shunji WATARI[**]

亘理俊次[**]：　石川県中新世産のアブラギリ属の化石木[*]

Recently the writer received a fragment of silicified wood, about $7\times10\times15$ cm in size, together with a set of ground sections from Mr. H. Matsuo of the Geological Institute of the Kanazawa University. According to him the specimen was obtained from a huge trunk more than one meter in largest diameter and at least five meters in length, and he also announced that it was found from a green tuff formation (Miocene) at Seryo, Oosugidani-mura, Nomi District, about thirty Km southwest from Kanazawa City, Isikawa Prefecture.

For precise identification, several additional sections were cut. The state of preservation of internal structures in these sections is found to be fairly poor, inflation, deformation or disorganization of elements being observed elsewhere. However small areas which retain a considerable detail of structures are scattered here and there in most of these sections, and after carefully examining through them the
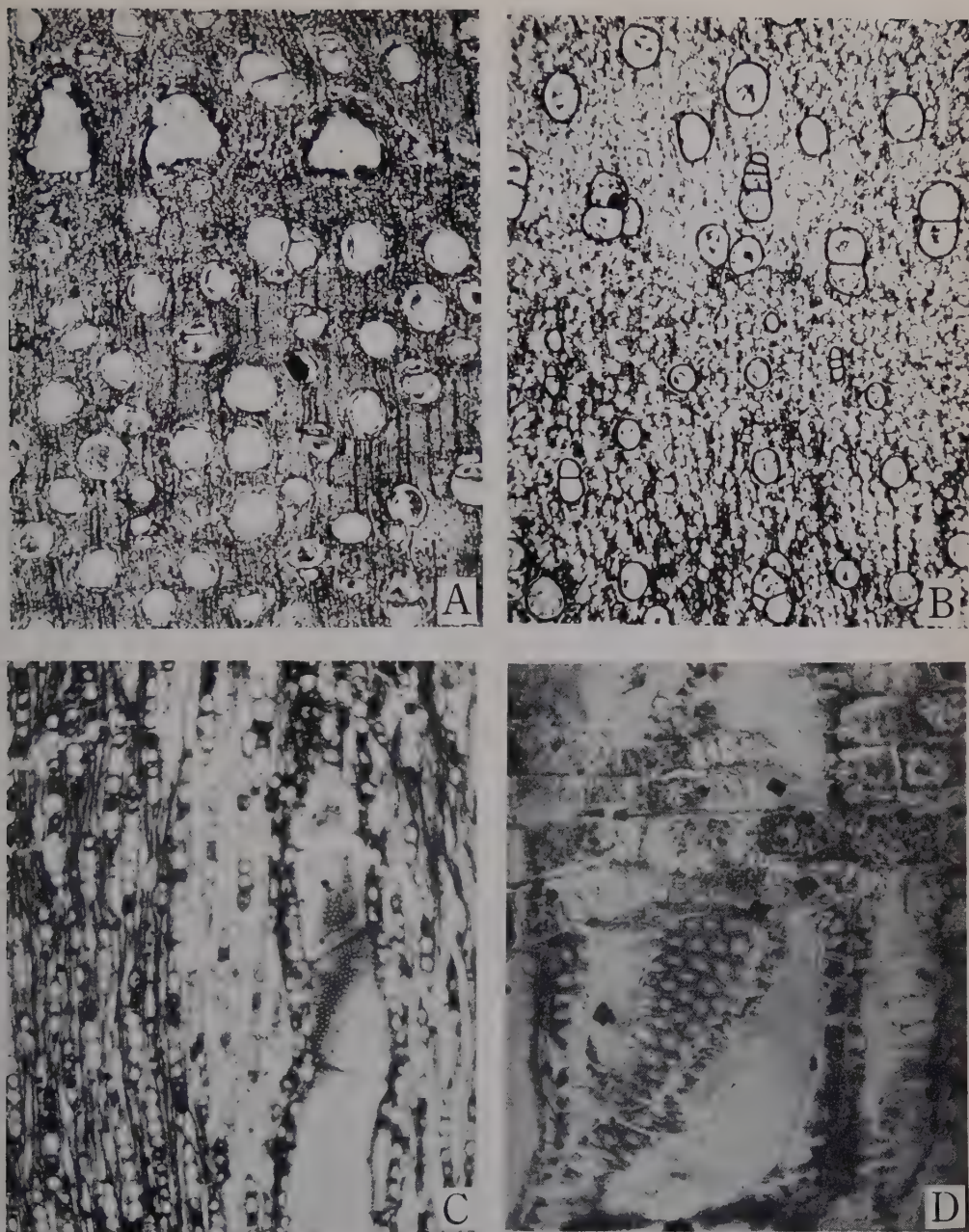

Fig. 1. *Aleurites miocenica* Watari, sp. nov.

A, cross section of a narrow-ringed specimen; three large triangular lumina on the upper part of the figure are traumatic secretory canals (×20).   B, cross section of a broad-ringed specimen showing the ring-porosity of the wood (×20).   C, tangential section showing a vessel and many uniseriate rays (×80).   D, radial section showing a vessel, a ray, and vasicentric parenchyma (×300; cf. Fig. 2).

writer came to the conclusion that the fossil in hand should be identified as an extinct species of *Aleurites* (Fam. Euphorbiaceae).

Aleurites miocenica Watari, sp. nov.

Description. Wood apparently ring-porous excepting narrow rings where this feature is more or less obscure (cf. note on p. 471). Boundaries of growth rings distinct but faintly, deliniated by a few layers of slightly flattened elements. Pores predominantly solitary and in radial multiples of 2-3 (occasionally up to several), or rarely in a netlike circular group or more or less irregularly clustered; solitary pores circular or oval in outline, the largest in earlywood 250–350 microns in tangential diameter; walls generally thin excepting the common-walls which are fairly thickened. Length of vessel segments 250–600 microns; perforation plates exclusively simple with more or less prominent perforation rim and slunting a ta variable angle; intervessel pits essentially alternate, circular to oval, or not infrequently polygonal through crowding; 6–15 microns in horizontal diameter, apertures lenticular to narrow elliptical and more or less horizontally placed, and 2–several inner apertures frequently confluent; thin-walled tyloses present in some vessels. Non-perforate tracheary elements forming the ground mass exclusively libriform fibers, non-septate, and arranged in more or less in regular radial rows, mostly 15–30 microns in diameter and very thin-walled. Parenchyma vasicentric and diffuse and slightly more developed in the porous zone; vasicentric parenchyma more or less sheathing the vessel, usually 1–2 layered around smaller pores, whereas in porous zone, occasionally aliform or rarely confluent; diffuse parenchyma scattered solitarily or in small groups among fibers; a parenchyma strand composed of 2–several thin-walled elements, individual elements measure 20–40 microns in diameter and 80–150 microns in vertical length; pits into vessel predominantly scalariform, half-bordered and provided with narrow aperture; chambered parenchyma containing figures suggesting the presence of solitary crystal rarely found. Rays unstoried and heterogeneous (Kribs Het. III), uniseriate or rarely
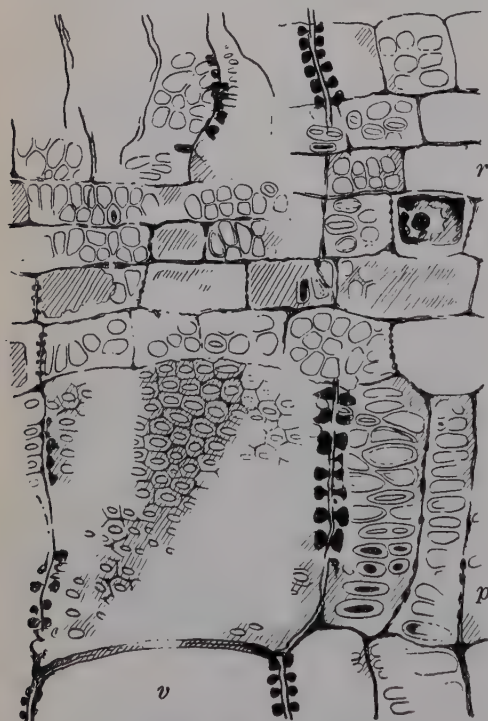

Fig. 2. *Aleurites miocenica* Watari, sp. nov. Redial section showing a vessel *v* with crowded polygonal pits, vasicentric parenchyma *p* with characteristic scalariform pits, and a ray *r* with various patterns of pits arrangement (×250).

in part biseriate, 1–20, mostly 3–10, cells high, consisting of a series of oval to circular cells, 15–40 microns in tangential diameter and provided with conspicuous triangular intercellular spaces; in radial section, it reveals that rays are composed exclusively of upright and squarish cells or interspersed by a few layers of short procumbent ones which correspond to the isodiametric elements in the tangential section. Excepting the part where a ray passes through the ground mass, upright elements 20–35, 40–60 microns, squarish ones, usually 20–40 microns, and procumbent ones 45–80×15–35 microns, in radial and vertical diameters respectively, whereas when a ray contacts with larger pores, individual elements more or less radially elongated. All walls slightly thickened and pitted; pits into vessel fairly variable in size, form, as well as in arrangement; circular to oval or angular by mutual contact and arranged in somewhat alternating rows in some places, and then, in other places, elongated or irregularly confluent to form various patterns; more frequently half-bordered with narrow lenticular to elliptical apertures, and occasionally simple. A tangential row of traumatic intercellular canals present in a cross section (No. 53202 A); canals circular, oval, or somewhat triangular in outline, 400–700 microns in tangential diameter, and spaced at 300–700 microns, no large pores being found between them; occlusion suggesting gummy substances present in every canals.

Note:——In a cross section (No. 53202 A) whose preservation is fairly excellent, growth rings are generally narrow, mostly 0.2-1 mm in width and faintly deliniated by a few layers of slightly flattened elements on the outer margin of increments. Most rings contain only a few layer of large pores or occasionally associate with an intermittent row of much smaller pores on the outer margin of increment, in extremely narrow ones there being only a single row of large pores. In other cross sections (No. 53202 D and E) whose preservation is extremely poor, it is observable that there are a few fairly broad rings (upto 5 mm) and pores are diminishing their size more or less abruptly toward the outer margin of increments. Thus it is apparent that the wood is essentially ring-porous and that the condition shown in the narrow-ringed specimen is caused by a lack of the typical zone of the latewood.

Locality and *horizon*: Seryo, Oosugidani-mura, Nomi District, Isikawa Prefecture; green tuff formation (Miocene); collected by H. Matuo in 1955.

Holotype; Botanical Institute, Faculty of Science, University of Tokyo, No. 53202, represented by nine ground sections (A-H).

Affinity. Important anatomical characteristics in the determination of the affinity of the present fossil may be summarized as follows: (1) ring-porosity of the wood; (2) fairly large, thin-walled pores which are scattered predominantly solitarily or in small multiples provided with more or less thickened common-walls; (3) simple perforation of vessel segments; (4) non-perforate tracheary elements forming the ground mass composed exclusively of non-septate, markedly thin-walled and large-lumened libriform fibers which indicates a soft and light nature of wood; (5) occurrence of diffuse and vasicentric parenchyma; (6) predominantly scalariform pits between parenchyma and vessel; (7) rare occurrence of the chambered parenchyma; (8) rays which are unstoried and exclusively uniseriate or rarely in part biseriate,

consisting of upright and squarish elements or interspersed by medial short procumbent ones (Kribs Het. III); (9) fairly variable patterns of pitting between ray and vessel; (10) occurrence of a tangential row of traumatic secretory canals.

Woods which are characterized by the occurrence of exclusively of uniseriate rays are known in many species belonging to a considerable number of families. Most of them, however, are easily eliminated by either of the following characteristics of them, that is, the scalariform perforation of the vessel (e. g. *Alnus*, *Hamamelis*, *Bucklandia*, *Cleyera*), perforation of vessel is simple but pores are markedly numerous or exceedingly small (e. g. *Populus*, *Salix*, *Zyziphus*, *Pyrus*, many species of Celarastraceae, *Aesculus*), and such special patterns of the pore distribution as radial, flame-like, or festoon-like arrangement (e. g. *Castanea*, *Castanopsis*, *Passania*, *Sideroxylon*). Heterogenity of the rays in the fossil further denies the affinity with such woods as *Tamarindus*, *Koelreuteria*, and *Pometia*. Thus there remain relatively a small number of woods whose rays are uniseriate and heterogeneous when one omits those mentioned above, that is, so far as the writer is aware, they are a certain member of the Euphorbiaceae (*Mallotus*, *Sapium* and *Aleurites*), Combretaceae (*Terminallia*), and Guttiferae (*Callophyllum*). However, *Sapium*, *Terminallia* and *Callophllum* are apparently distinguishable by their decided diffuse-porosity, the latter being also by the presence of broad bands of metatracheal parenchyma.

As is shown above, the predominantly scalariform pits between parenchyma and vessel makes one of the salient characteristics of the present fossil. This fasture was cited by Ogura (1932) in his study on a fossil species of the Euphorbiaceae, *Phyllanthinium pseudohobashiraishi*, as a feature which is observable only in the Euphorbiaceae and a few others such as Lauraceae, Anacardiaceae, Hamamelidaceae and Sabiaceae. In this regard, *Mallotus* is also apparently not the case, since in this genus the pits are circular and arranged alternately. It becomes hence obvious that there is none of woods otherwise a certain member of *Aleurites* which is perfectly identical with the combination of features listed above excepting the occurrence of traumatic secretory canals.

Recently, in his study on a fossil wood from the Tertiary of Sahara, Boureaux (1955) chose the occurrenc of the traumatic secretory canals as an important diagnostic characteristic and successfully arrived at the conclusion that it is an extinct species of the Combretaceae, *Terminallioxylon edengense*. Such a feature may, indeed, usefully and powerfully be utilized so far as this particular character is combined with an assortment of other characteristics which is common to the specimen under consideration and another fossil or living representative. It should be noticed here, however, that one scarcely expects a constant occurrence of such a sporadic feature in a given specimen as in the case of the present material in which only a single row of the traumatic canals is present among three cross sections, On the other hand, it is to be noted that there exist perhaps a considerable

number of woods whose possession of this character is unfortunately unrecorded or overlooked. In the present state of the wood anatomy of the dicotyledonous woods, our knowledge concerning to the said character may be somewhat different from case of some coniferous woods such as some members of the Pinaceae and Taxodiaceae whose traumatic reactions have been repeatedly discussed as an important item in considering their phylogeny.

According to Boureaux, this special traumatic reaction occurs in twenty-five families including the Euphorbiaceae and he noted that it is known in this family only in some species of *Croton*. As mentioned by him, there are no records of the presence of this character in the modern representatives of *Aleurites* and the writer also fails to find out it in *A. cordata*, yet it is not regarded, as discussed above, as a factor which denies the affinity of the fossil with the said genus, since other numerous characters strongly indicate it, but the occurrence of the character in an intimately allied genus *Croton* belonging to the same subfamily may rather be capable as a fact intensifying this possibility.

The genus *Aleurites* comprises of two deciduous species, *A. Fordii* Hemsl. and *A. cordata* R. Br., and three evergreen species, *A. montana* Wils., *A. moluccana* Willd., and *A. trisperma* Blanco (Pax & Hoffmann 1931). The pattern of pore distribution in the fossil immediately suggests a close affinity with the deciduous species[1]. However, the sheath of vasicentric parenchyma, especially in the porous zone, of the fossil is slightly thicker than in the case of modern species. In this connection, it will be appropriate to consider that it is an extinct form of the geuns which is very close either to *A. cordata* or *A. Fordii*.

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1) Accoding to Janssonius (1934), the wood of *A. moluccana* is apparently diffuse-porous, pores are scattered very sparsely throughout the increments, and, furthermore, rays are not exclusively uniseriate but they reach 2-3 cells in width.

Notes on Gametophyte and Young Sporophyte of *Angiopteris suboppositifolia* de Vries*

by Yoshitomo Nozu**

野津良知**: リュウビンタイの配偶体と若い造胞体について*

·Recently, in order to solve various problems of the morphology and phylogeny of ferns, important studies on the nature of gametophytes and young sporophytes are rapidly increased and studies on the gametophyte of the Marattiaceae have been given by such authors as Jonkman (1878, *Marattia* and *Angiopteris*), Farmer (1892, *Angiopteris evecta*), Campbell (1911, *Marattia*, *Angiopteris* and *Kaulfussia*), Land (1923, *A. evecta*), Sasaki (1928, *Archangiopteris Somai*), Haupt (1940, *A. evecta*), and Stokey (1942, *Marattia sambucina*).

In December 1955, the writer had an opportunity to collect a considerable number of prothallia and a large amount of fully matured spores of *Angiopteris suboppositifolia* de Vries at Yawatano, Izu Peninsula, Shizuoka Prefecture. Most of the prothallia collected there bear young sporophytes showing various stages of their development and some of them are provided with sexual organs. The spores were sown on Sphagnum in the greenhouse and fortunately they germinated.

The present study on the gametophyte and young sporophyte was made on the basis of the materials collected in nature as well as those obtained in culture. The development of the sexual organs was investigated from the materials embedded in paraffin after fixation with formalin-acetic-alcohol and sectioned by microtome.

Observation

In this species radial spores are predominate. They measure 40×50 microns in average size. In culture they usually germinate within two weeks and germination occurs in a split of spors coat. Chloroplasts may be seen in the first cell of gametophyte. When 1–2 cells are formed a rhizoid emerges from the ventral surface of the basal cell, and it is typically large unicellular and reddish brown or sometimes chocolate brown in colour from its very first appearance. When the filament becomes about 2–3 cells long, a longitudinal division begins in the cell just behind the terminal

cell (Fig. 3), though occasionally there is no
filament stage but such a division takes its
place in all cells immediately after the
germination. The thallus increases gradual-
ly its width by subsequent longitudinal and
transverse divisions (Figs. 4, 5). At the
time when the thallus grows nearly to the
stage shown in Fig. 5, an apical cell may
be found usually at a more or less lateral
position of the thallus. The apical cell under-
goes frequent divisions to form a cordate
thallus preceeding to its activity is replaced
by marginal meristems, though, under some
unfavourable conditions, a spatulate thallus
is occasionally formed (Fig. 6). The further
growth of the thallus is, however, approxi-
mately symmetrical, though an unusual
activity of the marginal initials occasionally

Fig. 1. Young sporophytes of *Angio-
pteris suboppositifolia* attached to the
gametophytes. ×1.5.

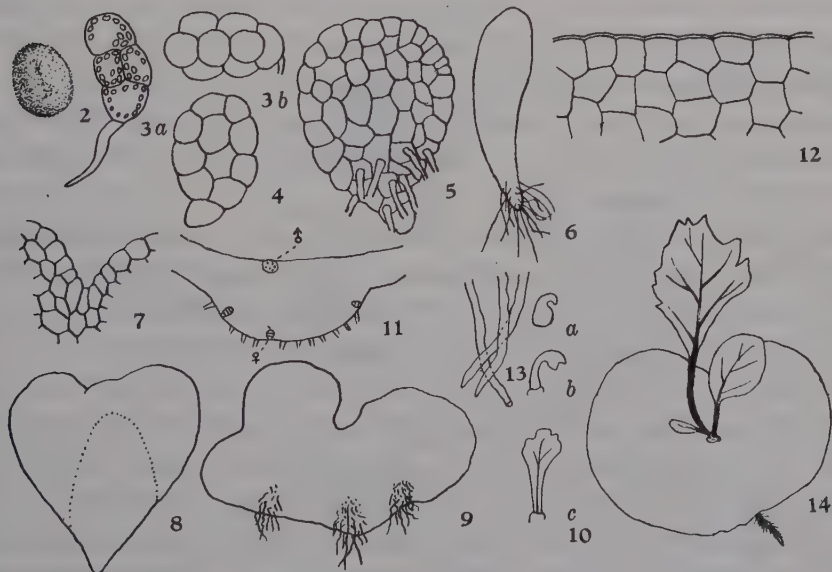
Fig. 2. Spore. Fig. 3. a, Beginning of germination (normal type). b, Massive one
(abnormal type). ×150. Figs. 4, 5. Development of gametophyte showing an apical
cell. Fig. 6. A case of spatulate form. Fig. 7. Detail of apical part of a young
gametophyte. Figs. 8, 9. Asymmetrical gametophytes. Fig. 10. a, b, c. Juvenile leaves.
Fig. 11. Cross section of the gametophyte showing the position of sexual organs.
Fig. 12. Marginal portion of gametophyte. Fig. 13. Apices of rhizoids. ×150. Fig.
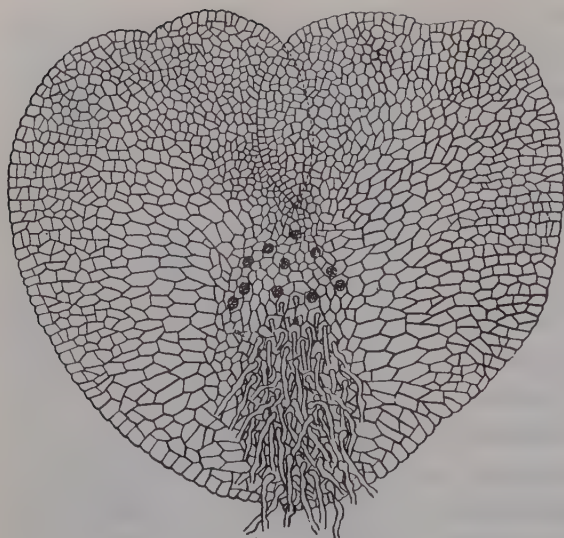14. Juvenile leaves attached to the gametophyte.

Fig. 15.　Detail of a mature gametophyte (ventral view).

results an asymmetrical form (Figs. 8, 9). In mature state (Fig. 15) the thallus assumes, alike the usual type of Polypodiaceous ferns, a broadly cordate form which is characterized with a deep notch and well developed wings composed of thin-walled cells containing numerous chloroplasts. The wing is of one cell in layer excepting the portion which is adjacent to the midrib (Figs. 11, 18). The fully grown prothallia bearing sex organs measure mostly from 5 to 10 mm both in length and in width (Fig. 15). But in the case of the older ones the wings are ruffling. Large numbers of unicellular rhizoids (Fig. 13) are found excepting the marginal portion. The marginal cells have no hairs (Fig. 15).

The midrib is about 10 cells thick and projected markedly on the ventral surface. Branching of the midrib is found on some asymmetrical gametophytes (Fig. 9). The gametophyte occasionally persists until the third leaf is fully matured. In culture, if conditions are favourable, a massive thickening of the thallus may occasionally occur at an early stage (Fig. 3, b). In this stage, a thallus is composed of about eight cells with chloroplasts.

Antheridium—Antheridium of the present species shows many particularities in its habit, form, as well as in its development. The antheridia occur usually on the dorsal side, rarely also on the ventral. The antheridium initial originates from the superficial cells of the thallus. It can occasionally be distinguished from the other superficial cells by its larger nucleus. The initial divides into the outer cell and inner cell by a periclinal wall. The outer of the two cells divides anticlinally, while the inner cell gives rise ultimately to the spermatogenous tissue. The two resulting outer cells then divide slightly oblique or anticlinally, namely, the primary cover cell undergoes three anticlinal divisions forming in the center a triangular opercular cell which is located at the more outer portion of the antheridia (Fig. 16). The inner cell is divided firstly by an anticlinal wall and subsequent divisions of these cells are simultaneous. The spermatocyte mass is cut off from the surrounding cells by a mantle or jacket layer, but it is sometimes incomplete (Fig. 17). At the later stage, the coiled spermatozoids are clearly visible in section. An antheridum may contain 30 to 60 sperms. Thus, the antheridium assumes a form of

sunken and large ball, there being a great range in their size.   Broken opercular cells are observed elsewhere, though the behaviour on the dehiscence of the antheridium was not observed.

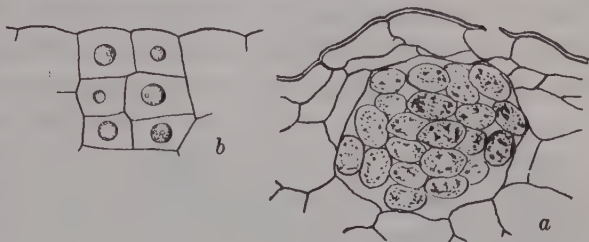Fig. 16.   Surface views of an antheridium and triangular cap cell. ×600.

Fig. 17. a, Longitudinal section of a mature antheridium. ×600.   b, young antherium. ×600.

Archegonium—The archegonium is always produced on the ventral side of the prothallium when it is about one month old in culture.   They appear in considerable numbers, but rather less than in most of the Leptosporangiate ferns.   The initial cell may be distinguished from the neighbouring superficial cells by its larger nucleus and the deeply stained cytoplasm.   The development of archegonium proceeds in the usual manner, as given by Haupt (1940).   Namely, the initial is divided into primary neck cell and the central cell by a periclinal wall.   The primary neck cell soon divides into two, then four by successive anticlinal divisions. Differing from the case of many ferns, neck of the archegonium does not project prominently from the surface, but bulges out a little above the surface.   In the meantime the central cell divides into two, a ventral cell and a neck canal cell, one of which, that is, the ventral cell, soon divides in the usual fashion again into a ventral canal cell and an egg.   The ventral canal cell relatively large, while the egg is rather small and lies at the bottom of
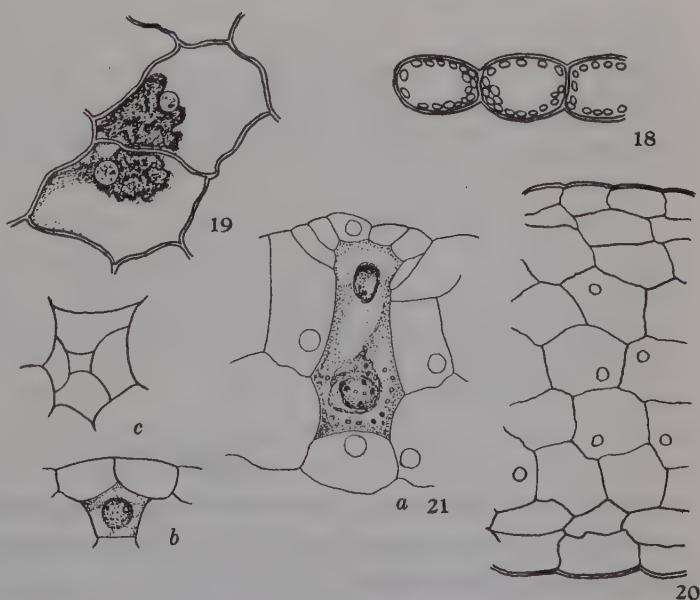

Fig. 18. Cross section of marginal portion of the gametophyte. ×150.   Fig. 19. Cells of midrib. ×600.   Fig. 20. Cross section of a part of midrib. ×600.   Fig. 21. Archegonium. a, Mature archegonium in longitudinal section. ×600.   b, Young archegonium in longitudinal section. ×600. c, Surface view. ×150.

the wide archegonial cavity. The neck canal cell is broad and divided into two, but usually the division wall may be found. The basal cell is not always found.

Young sporophyte—Earlier stages of the leaf development are shown in Fig. 10. At the one end of the embryo the divisions are more active and gradually the initiation of the stem and the primary leaf can be recognized. At a comparatively later stage the endogenous structure o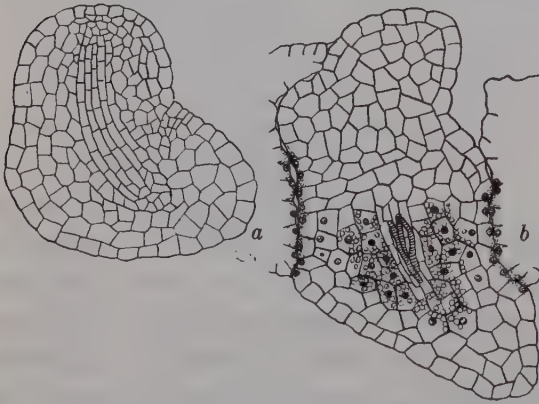f the primary root makes its appearance by the active cell divisions in the another end of the emdryo, and the root soon emerges on the lower side. The apical cell of the root is essentially tetrahedral, though in the later stages this condition is apt to be obscure. The derivatives of the apical cell appear by anticlinal divisions. The cortex is derived from the middle and to some extents from the inner and outer cells which resulted from the periclinal divisions of

Fig. 22. Longitudinal sections of embryos in advanced stage. × 150.

the cells adjacent to the apical cell. In the primary root can usually be found a centrally placed vascular bundle which is provided with two or three spiral tracheids. At this stage, tannin can be recognized in most cells of the cortex which are stained very strongly with safranin, starch grains being also found at the inner region of the cortex.

A primary leaf appears on the dorsal surface as a small protuberance as is shown in Fig. 10, a. A primary leaf may reach the length of 10 mm, and the lamina usually acquires the shape of a fan (Fig. 14), though the form is variable as reported by Campbell (1911). The venation of successive juvenile leaves changes from dichotomous to sympodial branching.

Discussion

There are notable differences between the morphological characters of the gametophyte of *Angiopteris* and those of the Polypodiaceous ferns. Those which are of special interest are:

a) particular feature in the form of the thallus in the early stage of its development

b) characteristic dark green colour of the thallus

c) markedly thickened midrib

d) absence of hairs

e) reddish brown, unicellular and large rhizoid

f) sunken and large antheridium

g) archegonium with less projected neck cells

h) small egg and wide archegonial cavity

After the germination of spore, the filament does not markedly elongate as in the case of many other ferns, but the short filament is soon transformed into a thin and more or less broad body, or, occasionally in culture, into somewhat massive one. It is interesting enough in this connection that these two conditions agree respectively with the "plate" and "mass" formation pointed by Stokey in an earlier stage of the prothallium development of such primitive ferns as *Marattia*, *Dipteris* and *Matonia* (1942, 1945, 1952). One of the characteristics of the genus, the strikingly deep green colour of the prothallia is said to be related to their long-lived habits as pointed out by Eames (1936) and Haupt (1953). The midrib of the prothallia in *Angiopteris* may reach about 10 cells in thickness. It also agrees with the case of primitive ferns such as *Matonia*, *Dipteris*, *Gleichenia* and *Cheiropleuria*. As Land (1923) pointed out, the markedly thickened midrib of *Angiopteris*, as well as of the other primitive ferns mentioned above may be capable as a feature which indicates a certain relationship with such ferns as Psilotaceae, Lycopodiaceae and Ophioglossaceae whose gametophytes are massive and subterranean in their habits. Furthermore, the absence of hairs excepting unicellular and large rhizoids in the gametophyte of this genus seems to be received as a characteristic of the primitive ferns.

The character of sex organs of *Angiopteris* is not found in any other ferns, excepting relatively few lower members. The antheridium in *Angiopteris* is of the sunken type with divided cap cells and the spermatocyte mass which produces 30–60 sperms. The development of the antheridium in *Angiopteris* resembles that of other members of the Marattiaceae and Ophioglossaceae (Campbell 1911, Haupt 1940 and Nozu 1954). Also a triangular opercular cell in the antheridium is a characteristic known only in a few of other ferns. So far as the writer's observation is concerned, a large numbers of archegonia are born only on the ventral surface, though Jonkman (1878) found them also on the dorsal side. The archegonium of this species is short and scarcely projects above the surface. So far as they are known, such a type of archegonium (also antheridium) is known only in some Eusporangiate ferns. In *Archangiopteris*, Sasaki (1928) found that the archegonia were situated near its median portion of the ventral surface of the thallus and took a small circular form. Ophioglossaceous ferns are example of such a case, but the archegonium neck of them is more slightly projected than in the case of *Angiopteris*. As Jonkman and Farmer illustrate, an archegonium is always project provided with a canal composed of two conspicuous neck canal cells, there being never observed a single binucleate neck canal cell which was found by Campbell (1911) in *Marattia*. The egg is small and lies at the bottom of the wide archegonial cavity, as pointed out by Haupt (1940) and Stokey (1942). In *Angiopteris* a basal cell in the archegonium does not always be found, as was reported by Farmer (1892) and Haupt (1940).

Though it is unnecessary to discuss an early development of the young sporophyte here, because Farmer (1892) and Campbell (1911) already reported in detail, it is to be noted that the tannin cells adjacent to the vascular bundle are conspicuous and the definite endodermis is not found.

As already mentioned above, most of characteristics of the gametophyte in *Angiopteris* are indeed primitive and show little affinity to other ferns. In regard to the question of the relationship of the Marattiaceae to Ophioglossaceae, Campbell (1911) states that they show a marked resemblance in many respects. However, in the writer's observation the gametophytes of both *Angiopteris* and Ophioglossaceae resemble each other in its antheridial characters but differ both in mature features and in archegonial characters, just as in the case of sporophyte.

Summary

The gametophyte and young sporophyte of *Angiopteris suboppositifolia* grown in nature and in culture were studied. Spores germinated about two weeks after, giving rise to a very short filament which develops soon to form a thin body or, rarely in culture, somewhat massive one. The fully mature thallus is characteristic dark green in colour and broadly cordate with a midrib which may reach about 10 cells in thickness. Excepting unicellular, large and reddish brown rhizoids, no hairs are present at any stage. Antheridium is situated on the dorsal surfaces, rarely also on the ventral; they are of the sunken and large primitive type with a triangular cap cell and a large output. Archegonium is situated always on the ventral surface; the archegonium neck is short and scarcely projects above the surface, and the small egg lies at the bottom of the wide archegonial cavity. In the type of germination and in the form of mature thallus, *Angiopteris* shows the primitive type of the Leptosporangiate ferns, while in the features of sex organs it resembles closely such Eusporangiate ferns as the other genera of the Marattiaceae and Ophioglossaceae.

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Quantitative und morphologische Studien über die ober- und unterirdischen Stämme von eingien Krautarten*

von Akira MIYAWAKI**

宮脇　昭**：　数種草本植物の地上茎と地下茎の形態についての量的比較研究*

Morphologisch sind oberirdische und unterirdische Stämme eigentlich das gleiche Organ. Der erstere wächst oberirdisch und der letztere unterirdisch. Funktionell trägt der oberirdische Stamm Blätter und Blüten, während der unterirdische Stamm den Obererdteil der Pflanze stützt, Wurzeln aus jedem Knoten treibt, und sogar die Nahrungsstoffe speichert. Anatomisch gesehen, gibt es im allgemeinen auch einen ziemlichen Unterschied in beiden Stammformen in bezug auf die Menge der Hauptgewebsteile. Jedoch ist bisher über die Messung vom oberirdischen und unterirdischen Stamm noch wenig berichtet.

Der Verfasser hat an oberirdischem und unterirdischem Stamm von einigen Krautarten einen quantitativen Vergleich des inneren Baues gemacht. Um einen Anhaltspunkt über die Ursache dieses Unterschiedes zu gewinnen, wurden die oberirdischen Stämme in die Erde begraben und andererseits die unterirdischen Stämme aus der Erde herausgegraben und beide weiter kultiviert. Die dadurch herbeigeführte Strukturveränderung in den Stämmen werden im folgenden im Vergleich zu intakten Stämmen berichtet.

Material und Arbeitsmethode

Von April bis August 1952 wurde eine Vorprüfung ausgeführt. Auf Grund der Ergebnisse sind weitere Versuche vom April bis August 1953 mit den folgenden Arten ausgeführt worden: *Mentha arvensis* L. var. *piperascens* Malinv. (Labiatae), *Houttuynia cordata* Thunb. (Saururaceae), *Aster Yomena* Makino (Compositae) und *Calystegia hederacea* Wall. (Convolvulaceae). *Mentha arvensis* wurde von Prof. Ogura verschafft und die übrigen Arten im Grundstück der Yokohama Staatlichen Universität gesammelt. Ökologische Behandlung ist im Versuchsgarten der Universität ausgeführt worden.

Aus dem Ergebnis der Vorprüfung, wurde einiger quantitativer Unterschied in demselben Individuum klar gesehen, je nach der Stufe des Wachstums und dem Teil der Pflanze. Die Versuchspflanzen wurden jeweils aus denselben Pflanzengesell-
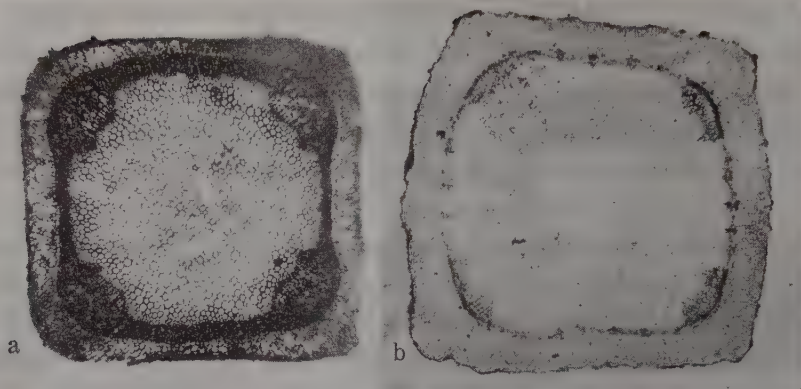
Abb. 1. Querschnitte durch Stämme von *Mentha arvensis*; a,
oberird. Stamm, b, unterird. Stamm. × 15.

schaften von ziemlich gleicher Individuendichtigkeit ausgewählt. Um die genauere
vergleichende Beobachtung zu machen, wurden Blöcke aus dem Internodium zwischen
4. und 5. Knoten jeder Pflanze herausgeschnitten, und aus diesen die Querschnitte
mit dem Mikrotom hergestellt. Alle Messungen wurden am Durchmesser des Stammes
und des Marks, und an der Dicke der Rinde und des Gefässbündelbezirks bei jedem
Schnitt mit dem Mikrometer gemacht. Ausserdem wurden **Rd–Q*** (das Verhältnis
von Dicke der Rinde zu dem Radius des Stammes) und **Mr–Q**** (das Verhältnis von
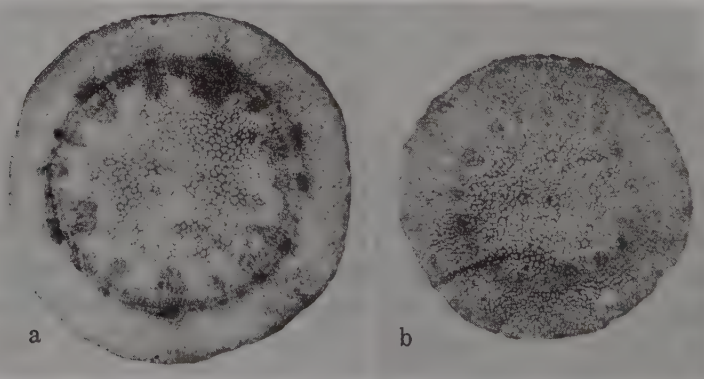Radius des Marks zu dem des Stammes) gerechnet.

Abb. 2. Querschnitte durch Stämme von *Aster Yomena*; a,
oberird. Stamm. b, unterird. Stamm. × 15.

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* **Rd–Q**. (=Die Dicke des Rinden-Quotienten) (Miyawaki 1955) stellt den Prozentsatz von der
Dicke der Rinde zum Radius des Stammes desselben Individuums dar; nämlich,

$$\mathbf{Rd\text{-}Q} = \frac{\text{Dicke des Rindes}}{\text{Radius des Stammes}} \times 100$$

** **Mr–Q**. (=Der Radius des Mark-Quotienten) ist ebenfalls;

$$\mathbf{Mr\text{-}Q} = \frac{\text{Radius des Marks}}{\text{Radius des Stammes}} \times 100$$

Das Experiment besteht darin, dass der oberirdische Stamm in den Boden be-
graben und der unterirdische Stamm dagegen aus dem Boden herausgenommen
wurde. Dieses Verfahren wurde an *Mentha arvensis* var. *piperascens* und *Hout-
tuynia cordata* angewandt. Nach 30 Tagen wurde die Messung ausgeführt und der
Vergleich mit den Kontrollen gemacht. Von allen Werten der Messungen wurden
der Mittelwert und die Standardabweichung berechnet und die Differenz der statisti-
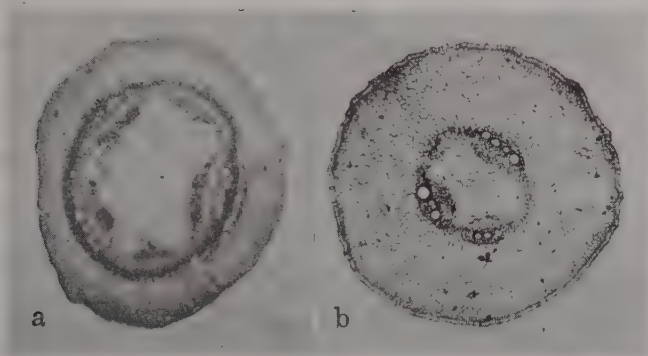schen Sicherheit mit dem Mittelwert ausgerechnet.

Abb. 3. Querschnitte durch Stämme von *Calystegia
hederacea;* a, oberird. Stamm, b, unterird.
Stamm. ×15.

Versuchsresultate und Diskussion

1. Der Vergleich des oberirdischen und unterirdischen Stammes.

Die Resultate der Messungen an den inneren Strukturen sind in der Tabelle 1
angegeben. Dazu wählten wir Stämme von beinahe gleichem Durchmesser. Es wur-
den Differenzen der statistischen Sicherheit in 3 Arten mit der Ausnahme von *Mentha
arvensis* bemerkbar. Nämlich waren die Durchmesser der oberirdischen Stämme bei
Houttuynia cordata und *Calystegia hederacea* kleiner als die der unterirdischen,
während die Sache bei *Aster Yomena* ganz umgekehrt war. Im Durchmesser des
Marks war der oberirdische Stamm grösser als der unterirdische. Die Dicke des
Gefässbündelbezirks verhielt sich wie der Durchmesser des Marks in *Houttuynia
cordata, Aster Yomena, Calystegia hederacea*, während die Sache in *Mentha arven-
sis* umgekehrt ist. Die Dicke der Rinde war bei oberirdischem Stamm kleiner als bei
unterirdischem von den 4 Arten, und zwar wurden die Differenzen der statistischen
Sicherheiten an 3 Arten, *Aster Yomena* ausgenommen, gesehen. Da aber in diesen
Fällen die Durchmesser der gemessenen Stammteile nicht gleich waren, ist das Ver-
hältnis des Radius des Stammes zu der Dicke der Rinde natürlich notwendig geworden.
Daher stehen die Resultate, die bei allen der 4 Arten durch **Rd–Q** berechnet und ver-
glichen wurden, in nahem Verhältnis mit der Differenz der statistischen Sicherheiten :

Tabelle 1.　Anatomischer Vergleich des oberirdischen und unterirdischen Stammes (Messung in Mikron).

		Mentha arvensis	Houttuynia cordata	Aster Yomena	Calystegia hederacea
Durchm. d. Stammes	Ober.	3163±454.8 (30)	2840±432.0 (50)	4005±600.2 (20)	2106±410.8 (30)
	Uts.	177	272	782	1001
	Unter.	2986±625.2 (30)	3112±422.3 (51)	3223±537.1 (31)	3107±724.6 (30)
Durchm. d. Marks	Ober.	2280±375.9 (30)	1844±435.0 (50)	1745±266.8 (20)	1003± 59.5 (30)
	Uts.	160	303	458	37
	Unter.	2120±364.0 (30)	1541±422.3 (51)	1287±313.6 (31)	966± 37.0 (30)
Dicke. d. Gef.	Ober.	197± 44.6 (30)	257± 35.4 (50)	510± 98.2 (20)	275± 53.1 (30)
	Uts.	19	15	173	10.3
	Unter.	216± 73.0 (30)	242± 52.8 (51)	337± 74.3 (31)	265± 86.6 (30)
Dicke. d. Rinde	Ober.	229± 39.7 (30)	250± 48.3 (50)	570± 12.5 (20)	268± 38.5 (30)
	Uts.	166	272	39	505
	Unter.	395± 52.6 (30)	522± 69.0 (51)	:609± 29.3 (31)	773± 23.0 (30)
Mr-Q	Ober.	71.7± 4.02(30)	63.96± 6.26(50)	44.1± 5.97(20)	45.7± 3.73(30)
	Uts.	8.4	14.91	5.0	13.2
	Unter.	63.3± 4.94(30)	49.05± 4.08(51)	39.1± 4.88(31)	31.5± 1.44(30)
Rd-Q	Ober.	14.9± 1.96(30)	17.52± 2.06(50)	28.8± 2.58(20)	27.2± 2.98(30)
	Uts.	9.66	16.28	10.2	23.8
	Unter.	24.6± 3.06(30)	33.8 ± 3.42(51)	39.0± 1.84(31)	51.0± 4.90(30)

Durchm. = Durchmesser, Gef. = Gefässbundel, Ober. = Oberirdischer Stamm, Uts. = Unterschied, Unter. = Unterirdischer Stamm.　Eingeklammerte Zahl zeigt gemessene Individuenzahl. Differenz der statistischen Sicherheit in Bedeutungsschwelle von 5 Prozent ist unterstrichen.
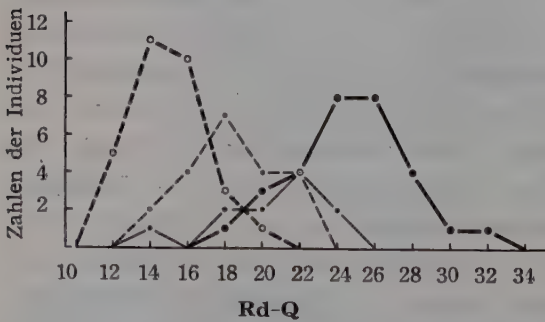

Abb. 4.　Vergleich der **Rd-Q** Kurven von *Mentha arvensis*.　Dicke ausgezogene Linie = unterird. Stamm, gestrichelte = oberird. Stamm, dünne ausgezogene Linie = herausgegrabener unterird. Stamm, dünne gestrichelte = begrabener oberird. Stamm. Individuenzahlen in Tabelle 1, 2 und 3.

d. h. oberirdischer Stamm kleiner als unterirdischer Stamm (Abb. 4—7). Es wurde gefunden, dass die Dicke der Rinde vom unterirdischen Stamm im Vergleich zu der des oberirdischen 1.6 mal als bei *Mentha arvensis* (Abb. 1), 1.4 mal bei *Aster Yomena* (Abb. 2) und 1.9 mal bei *Houttuynia cordata* (Abb. 8) und *Calystegia hederacea* (Abb. 3) grösser war. Mit anderen Worten, liegt der Gefässbündelbezirk der unterirdischen Stämme 1.4 bis 1.9 mal tiefer im Innern als in den oberirdischen.

Ganz umgekehrtes Verhältnis zeigen die Werte von **Mr–Q** bei 4 Arten; d. h. sie sind grösser im oberirdischen Stamm als im unterirdischen.
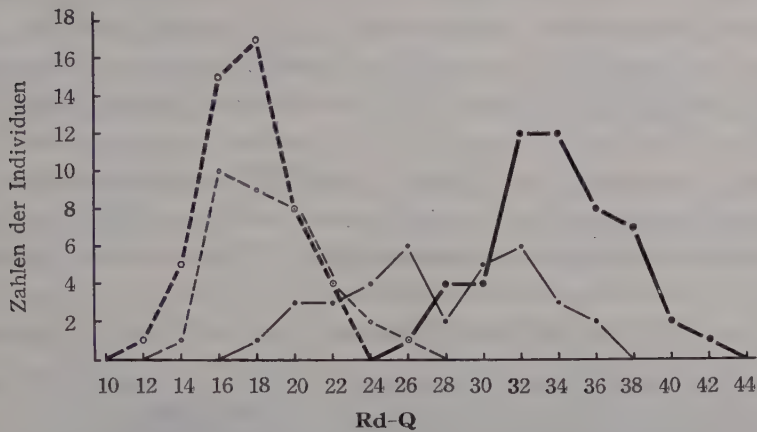

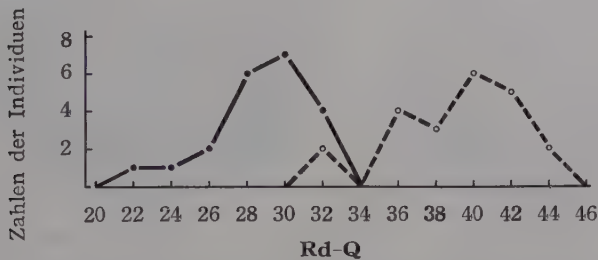Abb. 5. Vergleich der **Rd–Q** Kurven von *Houttuynia cordata.* Erläuterung in Abb. 4.

Abb. 6. Vergleich der **Rd–Q** Kurven von *Aster Yomena.* Erläuterung in Abb 4.
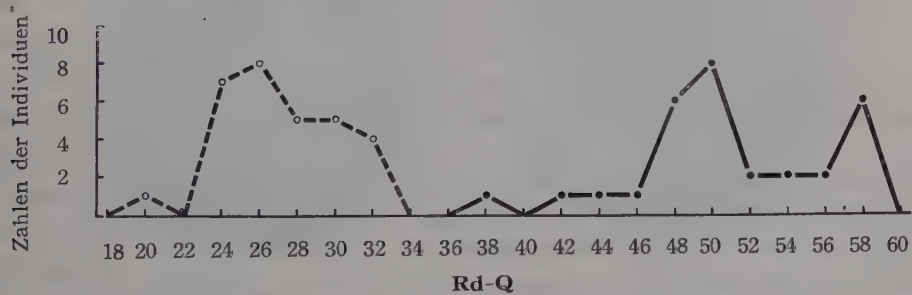
Abb. 7. Vergleich der **Rd–Q** Kurven von *Calystegia hederacea.* Erläuterung in Abb. 4.

2. Der Vergleich des begrabenen oberirdischen und des herausgegrabenen unterirdischen Stammes mit den Kontrollen.

Im Naturzustand ist die Dicke der Rinde beim unterirdischen Stamm im Gegensatz zum oberirdischen grösser: in anderen Worten tritt der Gefässbundelbezirk verhältnismässig innenseits zurück. Um die Ursache dieses Unterschiedes zu erfor-

schen, machten wir an dem Standort ökologische Experimente mit *Mentha arvensis*
(vom 18. Mai bis 17. Juni) und *Houttuynia cordata* (10. Juni bis 10. Juli), indem
oberirdische Stämme bis zum 6. Knoten in die Erde begraben und unterirdische
Stämme aus der Erde auch bis zum 6. Knoten herausgegraben wurden.　Nach 30
Tagen wurden die Internodien zwischen dem 4. und 5. Knoten zur mikroskopischen
Untersuchung herangezogen.　Im Aussehen liess sich kaum bemerkenswerter Unter-
schied beobachten, nur mit der Ausnahme, dass aus dem begrabenen oberirdischen
Stamm Wurzeln entstanden.　In Tabelle 2 sind die Vergleichsergebnisse mit den
intakten Stämmen angegeben.　Bei den begrabenen oberirdischen Stämmen steht der
Radius des Marks im allgemeinen proportional dem Durchmesser des Stammes (Abb.
8.)　Der Zentralzylinder und die Rinde sind dünner in dem intakten Stamm als in
dem begrabenen.　Die Differenz liegt im Rahmen einer statistischen Sicherheit.
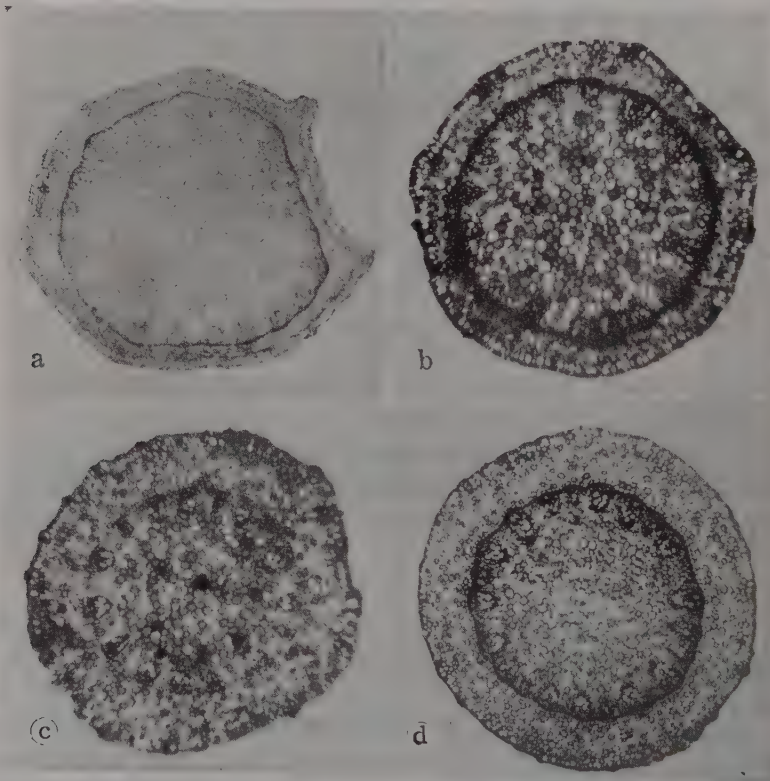
Abb. 8.　Querschnitte durch Stämme von *Houttuynia cordata;* a, intakter
oberird. Stamm,　b, begrabener oberird. Stamm,　c, intakter unterird.
Stamm, d, herausgegrabener unterird. Stamm.　× 15.

Die Werte von **Rd–Q** und **Mr–Q** sind in der Tabelle 2 und die erstere sowie
in Abb. 4 und 5 angegeben.　**Rd–Q** Werte sind kleiner in intakten als in den be-
grabenen und **Mr–Q** Werte umgekehrt.　Der Durchmesser des Marks bei intaktem
Stamm ist kleiner als bei dem begrabenen.

Der Durchmesser des Stammes im Verhältnis zu dem des Marks und zu der Dicke

des Gefässbündelbezirks ist kleiner in intakten als in herausgegrabenen unterirdischen. Jedoch an der Dicke der Rinde wurde ein umgekehrtes Resultat gewonnen (Tabelle 3). Dies ist noch sehr auffällig in bezug auf **Rd-Q**.

Aus diesen Ergebnissen ergibt es sich klar, dass sich die unterirdischen Stämme, die herausgegraben wurden, durch das Dickenwachstum während deren Behandlung histologisch den oberirdischen Stämmen nahegekommen sind, weil wegen der obengenannten Behandlung sich äusserliche Faktoren im Laufe von 30 Tagen veränderten; die begrabenen oberirdischen Stämme ähnelten den unterirdischen. Wieler (1891) hat als der erste darauf aufmerksam gemacht, dass der histologische Unterschied zwischen den Wurzeln, begrabenen und herausgegrabenen, in einiger Beziehung zu den äusserlichen zurückzuführen sei. Diesen Schluss hat er durch die Beobachtung, dass der in

Tabelle. 2. Anatomischer Vergleich der begrabenen oberirdischen mit intakten Stämmen (Messung in Mikron).

			Mentha arvensis	*Houttuynia cordata*
Duchm. d. Stammes	{	Intakt.	3163±454.8 (30)	2840± 432 (50)
		Uts.	118	650
		Begrab.	3281±630.8 (21)	3490± 498 (35)
Durchm. d. Marks.	{	Intakt.	2280±375.9 (30)	1844± 435 (50)
		Uts.	232	306
		Begrab.	2048±462.0 (21)	2150± 360 (35)
Dicke d. Gef.	{	Intakt.	197± 44.62(30)	257± 35.4 (50)
		Uts.	72	66
		Begrab.	269± 34.41(21)	323± 48.8 (35)
Dicke d. Rinde	{	Intakt.	229.3 ± 39.68(30)	250± 48.3 (50)
		Uts.	59.3	67
		Begrab.	288.6 ± 53.49(21)	317± 12.4 (35)
Mr-Q	{	Intakt.	71.7 ± 4.02(30)	63.96± 6.26(50)
		Uts.	7.4	2.13
		Begrab.	64.3 ± 6.84(21)	61.83± 3.82(35)
Rd-Q	{	Intakt.	14.9 ± 1.96(30)	17.52± 2.06(50)
		Uts.	3.7	1.28
		Begrab.	18.6 ± 2.4 (21)	18.8 ± 2.74(35)

Intakt.=Intakter Stamm, Begrab.=Begrabener Stamm. Andere Abkürzungen in Table 1.

Tabelle 3. Anatomisher Vergleich des herausgegrabenen unterirdischen mit intakten Stammes (Messung in Mikron).

			Mentha arvensis	*Houttuynia cordata*
Durchm. d. Stammes	{	Intakt.	2986±625.2 (30)	3112±422.3 (51)
		Uts.	505	114
		Herausg.	3491±468.5 (11)	3226±326.9 (35)
Durchm. d. Marks.	{	Intakt.	2120±364.0 (30)	1541±422.3 (51)
		Uts.	44	176
		Herausg.	2164±441.7 (11)	1717±930.2 (35)
Dicke d. Gef.	{	Intakt.	216± 73.0 (30)	242±52.75 (51)
		Uts.	84	53
		Herausg.	300± 69.15(11)	295±55.99 (35)
Dicke d. Rinde	{	Intakt.	395± 52.62(30)	522±68.99 (51)
		Uts.	18.7	87
		Herausg.	336.3± 78.19(11)	435±99.52 (35)
Mr-Q	{	Intakt.	63.3± 4.94(30)	49.05± 4.08 (51)
		Uts.	1.9	3.5
		Herausg.	61.4± 5.33(11)	52.6 ± 5.26 (35)
Rd Q	{	Intakt.	24.6± 3.06(30)	33.8 ± 3.42 (51)
		Uts.	4.0	6.2
		Herausg.	20.6± 2.92(11)	27.6 ± 4.92 (35)

Herausg.=Herausgegrabener Stamm. Andere Abkürzungen in Tabelle 1.

den Boden eingelegte Stamm Wurzeln austrieb, gezogen.　Fischer (1908) und Wolkott (1936) gaben an, dass eine quantitative Veränderung der Wurzel, die begraben war, von dem Einfluss des Lichtfaktors herrühren könne.　Morrison (1953) hat die Hypothese von Wieler und anderen über herausgegrabene oder begrabene Wurzeln von einigen baumförmigen Pflanzen beweisen wollen.　Aber er hat berichtet, dass er während einer Vegetationsperiode keinen eindeutigen Erfolg gewinnen konnte.

In diesem Versuche, in dem nämlich der oberirdische und unterirdische Stamm untersucht wurden, sind sie morphologisch eigentlich gleichwertig.　Der bemerkenswerte Unterschied war, dass im unterirdischen Stamm die Rinde dicker ist, indem der Gefässbündelbezirk verhältnismässig ins Innere verschoben ist und so weiter. Dieser Unterschied wurde nur in einer kürzeren Periode, also in 30 Tagen, durch eine einfache Behandlung hervorgebracht, woraus gefolgert werden darf, dass es sich hierbei um eine von äusseren Faktoren verursachte ökologische Erscheinung handelt.

Zusammenfassung

1.　Der innere Bau des oberirdischen und unterirdischen Stammes wurde an einigen Kräutern quantitativ untersucht.

2.　Die Rinde ist 1.4 bis 1.9 mal dicker in unterirdischem Stamm als in oberirdischem, und der Gefässbündelbezirk liegt im ersteren Fall also verhältnismässig tiefer im Innern des Stammes.

3.　Wenn oberirdische Stämme in die Erde begraben und unterirdische aus der Erde herausgegraben wurden und 30 Tage lang in diesem Zustande wuchsen, dann näherten sich die Werte **Rd-Q** von den ersteren Stämmen denen von den letzteren, und umgekehrt.

4.　Aus obigen Resultaten wird geschlossen, dass äussere Faktoren auf den inneren Bau der Stämme einen ziemlich starken Einfluss ausüben.

Literatur

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The Construction of Flowers under the Concept of Leaf-Class*

by Fumio MAEKAWA**

前川文夫**：葉類説にもとづく花の基本構成

Preface

The author has investigated over these ten years the phylogenetic development of vascular plants, especially the origins and evolutions of stem and leaves which are the most distinct organs in their somatic cormus or telomes, standing upon a new concept, the leaf-class concept quite different from any other already exsisted.

In this concept, it has been stressed that, 1) concerning the characters of many-sidedness, their coexistence to which many morphological concepts hitherto delivered, opposed or overlooked or neglected should be allowed to occur in the just same body or individual, and that 2) a organ might or might not be built up from a whole, i. e. it was sometimes constituted from some members of organs, which belonged to the lower order, and in some times, it was constructed only by some partial members of a whole organ in higher order.

The leaf-class G and the construction of F with S

The leaf is not the exception in these organs of manysidedness. The constructing members of leaf or leaves have been analyzed and named by the author, "Leaf-class" some of which were substituted not only to normal derivatives of leaves, but also to some unfamiliar ones ; integument of an ovule or to axis of an inflorescence.

Among several leaf-classes, the following three are remarkable in the modes or behaviours of their appearances, i. e. G, S, and F. The author has acknowledged some typical set of leaf-class G in Ginkgoariae as gymnospermous stage (1948, 1952) and also in Polycarpicaeariae as angiospermous one (1952), and called the group with G, as Phyllopsida in Cormophyta. To the other example of the pattern of appearance in leaf-class, the author discovered the synthetic pattern of two different leaf-classes, S and F, in Amentiferariae under angiospermous stage, i. e. the combination between the two members of the former in stipular phase and an individual of the latter in laminal or normal foliage leaf-phase. This group was named Stelopsida in Cormophyta.

　　The leaf arrangement (phyllotaxis) has received in different degree, evolutional change or transition. Though it is quite natural that their present stages are not always in the same order or level, the principal tendency of evolutional sequence is likely to be as follows:

　　Some irregular arrangement → (Adjustment of their position by the rythmical repetition in the establishment of leaf primordia with some intervals)

　　→ verticillate arrangement with over 3-leaves in each node → (the oligophyllous tendency in each stair of verticillate leaves till to reach the lowest number of their members, i. e. 2-leaves)

　　→ opposite phyllotaxis → (the dislocation of the setting position of leaves in longitudinal directions, leaving no change, in their relation to the divergency angle)

　　→ orixate phyllotaxis → (the farther coiling dislocation of leaves in every node)

　　→ perfectly alternate phyllotaxis.

　　These above mentioned sequences are different from the usually accepted one, even in the degree of quite opposite direction. But the author regards as true as such the general direction of the evolution in primary phyllotaxis both from the paleobotanical evidences–Cordaites (Maekawa, 1953, 1956) and ontogenetical development of several scales in bud or that of leaves in seedling stage, especially of Leguminosae.

The flower in general pattern

　　It is a usual acceptance that the flower is a much shortened stem along which many leaves in special floral form crowded in some regular orderiness. Indeed their constructions and appearances are very different and species – specific, but the fundamental general patterns too, may exist in their form and arrangement. These features are the five cyclic pattern of floral parts, i. e. sepal, petal, outer stamen, inner stamen, and carpel, counting from outer to inner one. Some one considers the carpel as fused one composed of the members in two cycles; outer sterile and inner fertile one.

　　Again in this cyclic arrangement, the generally accepted form of evolutional tendency is in the direction from 'alternate' of 'imbricate' in quinquncial pattern to 'valvate' or 'contorted' one; in the other words, these may be said in the tendency that from primitive 'alternate' to advanced 'verticillate.'

　　And in similar to the above mentioned manner in phyllotaxis evolution, the author accepts again the tendency from verticillate to alternate arrangement.

　　In the plant with alternate phyllotaxis of foliage leaves, while in its flower, with true cyclic arrangement, the latter arrangement should be interpreted as atavistic juvenile pattern induced by the sporophyll formation, rearly speaking, by influence through spore formation.

The flower composed of members of G - leaf-class

The typical pattern is showen in Fig. 1 with the example of perfect flower in pentamerous also pentacyclic one. From outer cycle to inner one there arrange in sequence of sepal, petal, outer stamen, inner stamen and carpel with marginal placenta. Many modifications, delivations, and reductions may occur in various taxonomical groups. The standard or the somewhat modified one can be found in Ranales.

As already pointed out by the author in the case of *Austrobaileya* (1952), each stamen can be accepted as the fusion of combined two members of G leaf-class, just as same as a seed-bearing pedicel of maiden-hair tree reduced to fuse into an organ, with its abaxial member of foliar phase on the short branch.

In some cases, the simple sporangiophore also may be acceptable, when the dichotomy does not occur in that stamen.

Thus these five members of each cycle are homologous each other in their standard construction and arrange in successively in regular alternancy. The sepal, outer stamen and carpel are combined into a set along one radius, while petal and inner stamen are composed in another set alternating to the former one; This relation is figured in Fig. 1 with zigzag broken line.

The flower composed of synthetic members of F and S

This pattern can be remarked in Rosales and Hamamelidales. In these cases, floral members are combined each other to make up groups which are governed under the law of alternation in their group level.

They are as follows:

$$
\left(
\begin{array}{c}
\text{Petal} \\
\text{Sepal} + \text{Outer stamen} \\
\text{Petal} \\
\\
\text{Outer group}
\end{array}
\right)
\left(
\begin{array}{c}
\text{Sterile carpel} \\
\text{Inner stamen} + \text{Fertile carpel} \\
\text{(Placenta)} \\
\text{Sterile carpel} \\
\text{Inner group}
\end{array}
\right)
$$

For the sample, the outer group may be taken. Sepal and outer stamen are both real members of F leaf-class, while two petals, the members of S leaf-class respectively.

The outer F corresponds to the leafy phase of a foliage leaf, as same as in the nodal arrangement, and the inner one, the sporangiophore or sporangial branch of axillary orientation to the said foliage leaf, i. e. the sepal.

They sprang out from the same nodal part, immediately divided into two strands arranged in a radial plain.

Petals, usually very different from sepal, in their texture and arrangement of nervation, belong to S leaf-class, and in the compared nodal arrangement, they correspond to two stipules in the two lateral sides of a foliage leaf.

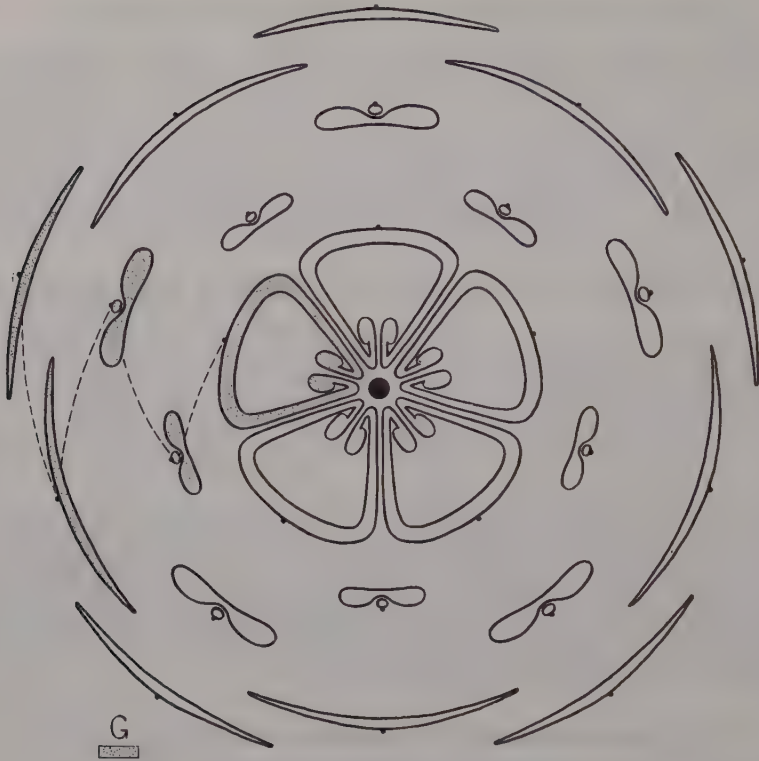Thus the reader can imagine that the outer group, composed of two members

Fig. 1. Floral diagram in the group composed of G leaf-class. Explanation in text.

of outer-inner F and two members of lateral S, is same to the laminal leaf F with one sporangial branch in its axil and two stipules in their lateral sides.

The inner group is alternate with the outer group. Concerning to its composition the standard pattern is the same as in the outer one. In this case, carpellar walls are realized as the sterile outer carpels, while placentas are combined each other into a set, which is stretched over the marginal end of sterile one. These fertile carpels are in set with inner stamen, just as same in the relation between an outer stamen and a sepal. To the lateral sides of these inner stamen—fertile carpel sets, two sterile carpels lie in side, the pattern of which is very much like the petals concerning to sepal—outer stamen set.

Summary

1. The floral constructions are considered under the concept of leaf-class.

2. In seed plants, there are, at least, two different patterns of floral construction. The one is SF-combination and the other, G-leaf-class.

3. The former is made up of two groups, the outer one is composed of 2-F (sepal and outer stamen) and 2-S (two petals). While the inner one, with 2-F (inner stamen and fertile carpel) and 2-S (two sterile carpels).

4. In the flower of G-leaf-class, floral leaves are of all G, arranged in pentacyclic
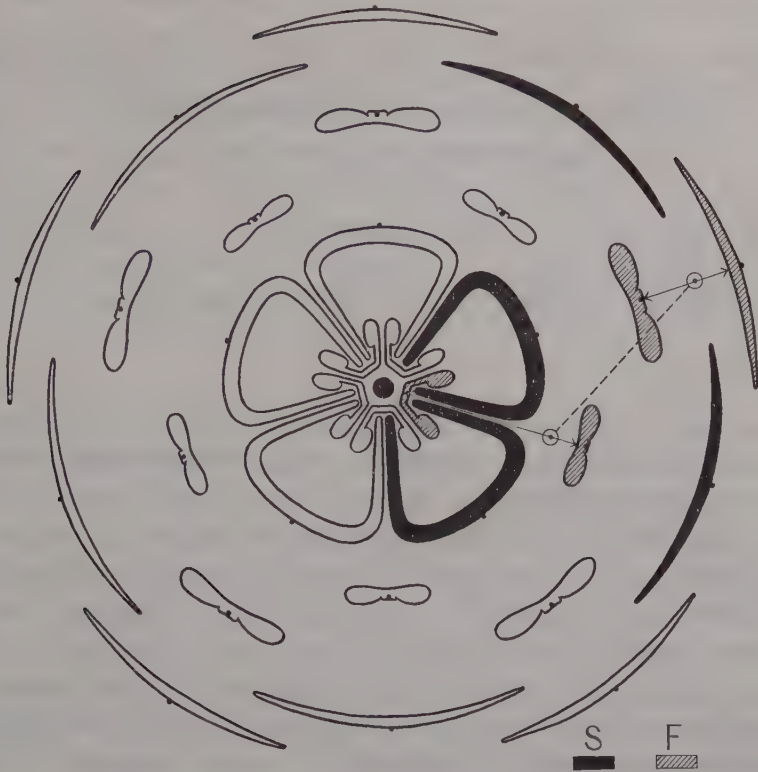
Fig. 2. Floral diagram in the group composed of S and
F leaf-class. Explanation in text.

manner with alternation of one radius with three member of sepal—outer stamen—carpel and the other, with petal and inner stamen.

5. Most types of different flower are in species-specific pattern but can be interpreted as the derivatives modified through several different courses from the above mentioned fundamental architectures.

Acknowledgement

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Determination of Electric Charge and rH by Means of the Staining Methods in Prothallia, Especially in Spermatozoids of Ferns

by Isami IGURA*

伊倉伊三美*: 染色法による羊歯類前葉体特に精子の荷電と rH の測定

Joyet-Lavergne (7, 8) proved by the staining method that the *Equisetum*-spores were divided in two types, male and female, whose oxidation-reduction potentials (rH) were different from each other. Kuwada (10, 11) studied also by the staining reaction the electric charge and obtiained results that the charge differed between the spermatozoid and the egg cytoplasm in *Cycas revoluta*. By means of the staining reaction of Joyet-Lavergne or Kuwada, Abe (1) confirmed the different charge or rH between the two kinds of gametes in five species of Myxomycetes. Since then the Keller's study (9), the charge or the rH has been studied by the staining reaction, but no investigation has been found concerning the spermatozoid (spermatid, antheridium), the egg (archegonium), or the prothallium of fern from the view-point of charge or rH.

The writer intended to make clear, by means of the staining reaction, the electric charge or rH of the prothallium, especially of the spermatozoid and the egg *in situ* or in fixed state. The present report describes the results of this study.

Material and methods

The following prothallia of ferns belonging to Eufilicineae which were cultured in the writer's laboratory were used as materials:*Athyrium pycnosorum* Christ, *Athyrium yokoscence* Christ, *Dryopteris crassirhizoma* Nakai, *Rumohra mutica* Ching, *Spicantopsis niponica* Nakai var. *japonica* Nakai, *Thelypteris japonica* Christ, and *Osmunda japonica* Thunberg. The spermatozoids produced on these prothallia were employed. In order to survey the electric charge, the staining reaction of Unna-Golodetz's neutral violet extra by the method of Keller and Kuwada and the prussian blue reaction were used. To make clear the oxidation color or the redution one the method of Mn-methyl green staining and the staining with rH-indicators were applied. The spermatozoids which were in- or outside of the spermatids and the egg cells in the archegonia were examined both in vital and fixed (with Carnoy's

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* Biological Institute, Faculty of Education, Yamagata University, Yamagata Pref., Japan.
山形大学教育学部生物学教室

fluid (3:1)) states.   Neutral violet extra was prepared by mixing of neutral red 0.5 g and new blue 0.25 g (neutral red: new blue$\fallingdotseq$2:1, by Kuwada) and redistilled water 75 cc, and the staining procedure was as follows: neutral violet extra (10–20 mins., occasionally 2, 3 hours)$\rightarrow$30% $\rightarrow$ 50% $\rightarrow$ 70% alcohol (1–3 hours) $\rightarrow$ sometimes 85% $\rightarrow$ 95% alcohol (each 10 mins. or 2 hours).   The cathode procedure of the prussian blue reaction was as follows: 10% $FeCl_3$ (10 mins.) $\rightarrow$ Washing $\rightarrow$ 10% $K_4Fe (CN)_6 \rightarrow$ glycerol (10 mins.), and the anode procedure was the reverse of the cathode one. The Mn-methyl green staining was made as follows: 1% $KMnO_4 \rightarrow 0.5N$ HCl $\rightarrow$ 1% methyl geen (each 10 mins.).

Results

(1) **Staining reaction with Unna-Golodetz's neutral violet extra.**
a) **Vital staining.** When the prothallia were immersed in the solution of neutral violet extra for ca. 10 minutes, the cytoplasm (perhaps, membrane too) of rhizoidal cell took the purple red dye and especially the young rhizoidal cells were stained deeper in its tip portion than the basal one, and this fact was considered to show the polarity of rhizoidal cell. Some membrane of the prothallial cells also took the same purple red dyes with the exception of the meristemal portion.

The spermatozoids included in the spermatids or extruded out of them took the purple red dyes and were stained. Of the structural elements of the spermatozoid-body, the elements which were stained were not the cilia-bearing band, the cilium, and the border-brim, but the nuclear portion.

When the spermatozoids (spermatids) were observed after being immersed in 30% and 50% alcohol, they showed almost same tones of the colors as stated above. However, when they were dipped in 70% alcohol for 20 minutes or more, sometimes for 1 hour their colorations gradually changed from the greenish blue into the blue.

The prothallium sometimes adsorbed the purple red dye, which resolved into the alcohol solution when it immersed in the graded alcohlol as above-mentioned and it did not show the blue color, while the rhizoidal cell remained occasionally purple red.
b) **Fixed material.** The results were similar to these with vital staining.   The materials were observed after they were immersed in the solution of Unna-Golodetz's neutral violet extra and washed and placed in 50% alcohol.   The spermatozoids included in the spermatids or extruded out of them took weakly the purple red dye or the red one.   The nucleus and the membrane of prothallial cell took considerably the purple red dye and the cytoplasm or the plastids also were stained slightly. The rhizoid and the glandular hair (cytoplasm, nucleus) showed often the same color too. The membrane, nuclei, and nucleolei of the cells at the meristem and its neighbourhood showed violet coloration and the color turned gradually to purple red as they approach towards the basal pole, therefore the prothallium was con-

sidered to show the gradient concerning the adsorption of dye.

When immersed in the 70% alcohol solution, however, for 2-3 hours or in the 80% alcohol for ca. 10 minutes, the spermatozoid-nucleus and the spermatid-cytoplasm changed their colors into blue. The chromatin in the spermatozoid-nucleus was considered to adsorb the blue dye. The cells in the meristemal portion of the prothallium and the apical cell of the protonema showed blue coloration and the nuclei of prothallial cells were also blue and towards the basal pole the color became weak purple red (Fig. 1). In archegonia which were not yet fertilized yet, the nucleus, the membrane, and the cytoplasm of neck cell assumed weak purple blue coloration and the egg seemed to show the purple red.

The same procedure was applied to the spermatozoids fixed by drying the drop of redistilled water in which they came out of the antheridia and were swimming about on the slide-glass. In this case, the sufficient and good result could not be obtained, but when dipped in 95% alcohol for about from 40 minutes to 2 hours, they seemed to become blue very weakly.

In both vital and fixed materials, the spermatozoid-nucleus and the spermatid-cytoplasm gave the staining reactions of blue. It was an interesting fact that, in the case of fixed material, the meristemal portion of the prothallium was blue and the color of the prothallial cell changed into purple red towards the basal pole. This fact might show the existence of the gradient (polarity) in the area of the prothallium.

　　(2) **Mn-methyl green staining.**

a) **Vital staining.** The nuclei of spermatozoids were in- or outside of the spermatids and the cytoplasm of spermatids were stained green or bluish green by the method of Mn-methyl green staining. The cytoplasm of rhizoidal cell or glandular hair of the prothallium adsorbed the brown or violet dye, and the meristemal portion usually took the bluish green dye.

b) **Fixed material.** The staining reactions of spermatozoid-nucleus and spermatid-cytoplasm were same as in the case of vital ones. As to the prothallium, the cellular elements of prothallial cell in the meristemal portion were green, but those in the other portion were brown in the whole area of the prothallium, or occasionally were locally brown, green, or violet. However, the localization of these colors was not considered to be regular, in other words, no gradient of the staining reaction in the area of prothallium seemed to exist. The rhizoidal cell showed often the brown (or violet) color and its tip the green color, so this fact showed the polarity of the rhizoidal cell.

A drop of redistilled water in which the spermatozoids swam about was dried up in room temperature. With these dried spermatozoids this Mn-methyl green staining method was tested. The spermatozoid-nuclei assumed at first $KMnO_4$ slightly and presented the weak yellowish brown colors, but discolored in 0.5 N HCl and at last their colorations appeared in green or purple green, assuming methyl

green (Fig. 2). The cilia-bearing band, the cilium, the border-brim of spermatozoid-body were negative to this reaction and these did not show the green color.

(3) Prussian blue reaction.

a) **Vital material**. Cathode procedure. The spermatozoids and the cytoplasm in spermatids were stained strongly in greenish blue *in vivo* (Fig. 3). The same phenomenon was seen in the rhizoid and the glandular hair. The tip of rhizoidal cell was more or less strongly stained in greenish blue. From this fact the rhizoid was considered to possess the polarity. Anode procedure. Some of the rhizoidal or the prothallial cell (membrane) showed very weak greenish blue, whereas the spermatozoid and the cytoplasm in spermatid were not stained.

b) **Fixed material**. Cathode procedure. The spermatozoid-nucleus, the spermatid-cytoplasm, the rhizoid, and the glandular hair which were fixed with Carnoy's fluid (3:1) presented the same result as in the case of vital ones. The portion near the meristemal one was not stained and the other prothallial cells were not stained or stained in blue locally. This procedure was tested to the spermatozoids also which just came out of the spermatids, and this result was same as in the case of the spermatozoids which were yet in the spermatids, as above-mentioned. Namely, the spermatozoid presented the yellowish brown color at first, and then turned into weak blue. Anode procedure. The spermatozoid and the cytoplasm in the spermatid were not stained. In prothallium, the meristemal protion was stained often in
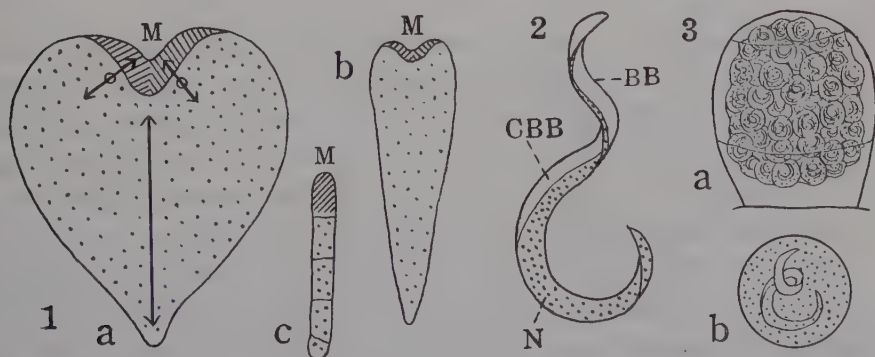
Figs. 1–3. The staining reactions of spermatozoid, spermatid, and prothallium which were drawn somewhat schematically.

1. Unna-Golodetz's neutral violet extra staining. Prothallium of *Dryopteris crassirhizoma* Nakai. ▨ Blue portion; ▦ Weak purple red portion (often violet, brown, or green color was shown locally). a. Mature prothallium (×ca. 10); b. Young prothallium (×ca. 10); c. Protonema stage (×ca. 15); ⟵⟶ Longitudinal polarity; ←⊖→ Radial polarity; M, Meristemal portion.
2. Mn-methyl green staining. Spermatozoid of *Dryopteris crassirhizoma* Nakai whose cilia are not shown (×ca. 750). ▦ Green or purple green portion. BB, border-brim; CBB, cilia-bearing band; N, nucleus.
3. Prussian blue reaction (cathode procedure). ▦ Greenish blue portion. a, Spermatids in the antheridium of *Spicantopsis niponica* Nakai var. *japonica* Nakai (×ca. 240); b, Spermatid (×ca. 450).

greenish blue slightly, while the other prothallial cells stained or unstained locally and this staining seems to have no regularity. The rhizoidal cells were unstained.

(4) The staining reactions with rH-indicators.

The tone of the color and the supposed rH of spermatozoid and rhizoid of the prothallium obtained by cresyl blue and other four kinds of rH-indicators are shown in Table 1. As to the rH-values the further studies will be made in future.

Table 1. Staining reactions and rH of spermatozoid, spermatid, and rhizoid of the prothallium.

Per cent of the indi- cators	Indicators	Spermatozoid-nucleus and Spermatid-cytoplasm			Rhizoidal cell		
		Vital	Fixed	Supposed rH	Vital	Fixed	Supposed rH
0.02	Cresyl blue	Blue	Greenish blue	Ox*	Bluish violet	Bluish violet	?
〃	Methylene blue	Blue or bluish green	Blue or bluish green	Ox	Green or blue	Green or blue	Red*
〃	Neutral red	Orange red	Orange red (weak)	Ox	Reddish brown	Reddish brown	Red
〃	Safranin	Orange red	Orange red	Ox	Orange red	Reddish orange	Vital Ox Fixed Red
〃	Thionin	Blue	Reddish blue	Ox	Reddish violet	Reddish violet	Red

* Ox, Oxidation; Red, Reduction

The staining reactions of spermatozoids, spermatids, and rhizoidal cell were almost the same both in the vital or the fixed state. Of the structural elements of the prothallium, the rhizoidal cell always took homogeneously these indicators in both vital and fixed materials and the other elements usually adsorbed the indicators *in vivo* (5), and in fixed state these elements sometimes adsorbed these dyes. For instance, when the fixed prothallia of *Thelypteris japonica* Ching were stained with the 0.02% redistilled water solution of thionin, the membrane of prothallial cell was stained fairly good in reddish violet and the cytoplasm also weakly in the same color (reduction color), but the nucleus in blue (oxidation color) and the meristemal portion in green. The prothallium was often stained locally without showing the regularity.

According to Table 1, the spermatozoid-nucleus or the spermatid-cytoplasm is considered to be the oxidation-portion and the cytoplasm of rhizoid the reduction-one.

Discussion and conclusion

In the solution of Unna-Golodetz's neutral violet extra the nuclear portion of spermatozoid and the cytoplasm of spermatid showed at first the purple red color but became blue, after taking alcohol. Of the spermatozoid-body, the cilia-bearing

band, the cilium, and the border-brim did not show this reaction. The change of the tone of color was as follows: purple red → violet → greenish blue → blue. The mature rhizoidal cell of the prothallium took at first the purple red dye and kept often this coloration even in the alcohol solution, but the young rhizoidal cell is stained weak blue. The cells of meristemal portion of the prothallium or the protonema became blue, and as near the basal pole, the color of prothallial cell was purple red. Therefore, concerning the adsorption of the dye, it is thought that the gradient exists in the area of prothallium. The nuclei of prothallial cells were blue. Thus, if we recognize that the staining reaction is interpreted by the electric charge both in the case of vital and fixed states, the following explanations of charge phenomena are supported from the results of the staining reactions stated above. That is to say, the nucleus of spermatozoid and the cytoplasm of spermatid have the negative charge somewhat strongly, and the distributions of the charge in the area of prothallium show that the meristemal portion is negatively charged and the other prothallial cell and the mature rhizoidal cell are positively charged. The young rhizoid and the nucleus of the prothallium are negatively charged.

The spermatozoid-nucleus and the spermatid-cytoplasm which were *in vivo* or fixed were stained greenish blue somewhat strongly by the cathode procedure in prussian blue reaction, while they did not show any colorations by the anode procedure, therefore they are negatively charged. This result agrees with that of the method of Unna-Golodetz's neutral violet extra. Supposing from the staining reactions of the prothallium, the mature rhizoid has the negative charge and the prothallial cells may have the positive or may have the positive and the negative charges locally, whose distribution on the prothallium is not regular.

Prussian blue reaction is applied to detect Fe^{3+} (2) and this is a reaction caused by the binding of Fe^{3+} and $[Fe(CN)_6]^{4-}$. The chromatin of the spermatozoid-nucleus is thought to adsorb easily Fe^{3+} in the cathode procedure, so that the prussian blue reaction occurs nicely. Swift (12) stated that the acid and the basic dyes are adsorbed in correlation with the charge of protein or nucleic acid and, the writer considers, the coloration of spermatozoid-nucleus which shows the negative charge is related to the contents of protein (nucleoprotein) or DNA (4) in the spermatozoid-nucleus.

By the method of Mn-methyl green staining, the nucleus of spermatozoid and the cytoplasm of spermatid are stained green or bluish green clearly. In the whole area of prothallium, the meristemal portion is green and other prothallial cells are brown but often brown, violet, or green color scatters. The cytoplasm of rhizoidal cell is stained brown, but that of tip of the rhizoid green. This fact may indicate the polarity of rhizoid. The rH can be supposed from the stand-point of the staining phenomena as stated before, and the spermatozoid-nucleus and the spermatid-cytoplasm are thought to be the oxidation portions. In prothallium, the meristemal portion is the oxidation portion and the other prothallial cells are reduction one or

have both oxidation and reduction portions. There are no regularity concerning the distribution of the oxidation and reduction portions. The rhizoïd is considered to be the reduction portion but the tip is the oxidation one and presents the polarity. Thus, the rH of spermatozoid-nucleus or the spermatid-cytoplasm is supposed to be different from that of the prothallial cell.

The results obtained from the staining reactions by rH-indicators support the following discussions. In the nucleus of spermatozoid and the cytoplasm of spermatid and rhizoid, almost the same results were obtained in both cases of vital and fixed materials. The former two show the oxidation colors and their rHs are high, while the latter one reduction color and its rH is low. The cytoplasm and the membrane of prothallial cells present the reduction colors, and the nuclei the oxidation ones, but often the localization of the oxidation and reduction colors were seen. And the difference of rH is found between the cytoplasm and the nucleus in prothallial cells.

Summary

To make clear the electric charge and the rH, the staining reactions by the methods of Unna-Golodetz's neutral violet extra, prussian blue, Mn-methyl green, and rH-indicators were tested to the spermatozoid (spermatid) or the prothallium in fern. The following facts were gotten from the results of staining reactions.

(1) The nucleus of spermatozoid shows the negative charge but the cilia-bearing band, the cilium, and the border-brim do not show it. The cytoplasm of the spermatid also show the negative charge.

(2) In the prothallium, the cells of the meristemal portion and the nucleus of prothallial cell, and the rhizoidal cell present the negative charges, whereas the cytoplasm of prothallial cells show the positive charge, or the posive and the negative charges locally. As to the charge, the polarity is thought to exist in the whole area of prothallium.

(3) The nucleus of spermatozoid and the cytoplasm of spermatid show the oxidation color and their rHs are high. In the prothallium, the meristemal portion presents often the oxidation color, whereas the prothallial cell (cytoplasm, membrane) and the rhizoid (cytoplasm) show the reduction color and their rHs are low. Judging from these facts the polar property of the prothallium is thought to exist.

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ミツイシコンブの游走子嚢発生と游走子形成
（コ ン ブ 目 の 形 態 発 生 学 的 研 究 I*）

西 林 長 朗** ・ 猪 野 俊 平**

Takeo NISHIBAYASHI** and Shumpei INOH**: The Development of
Zoosporangia and the Formation of Zoospores in *Laminaria angustata* Kjellm.
(Morphogenetical Studies in Laminariales I*)

コンブ目の游走子嚢の発生に関しては，Thuret (1850), Setchell (1891), Schrader (1903), Sauvageau (1918), Kylin (1918), Mckay (1933), Herbst and Johnstone (1937), Clare and Herbst (1938), Hollenberg (1939) らにより，種々の種について研究されているが，我が国の種については，未だ全く観察が行われていない。また，この目についての細胞学的研究には，古くは，Kylin (1918) の *Chorda Filum* (L.) Lamour, Myers (1928) の *Egregia Menziesii* (Turn.) Aresch., Mckay (1933) の *Pterygophora californica* Ruprecht, Hollenberg (1939) の *Eisenia arborea* Aresch. に関する報告が見られ，最近では，Walker が *Macrocystis integrifolia* Bory (1952), *Laminaria digitata* Lamour (1954). *Laminaria Cloustoni* (unpub.), Magne (1953) が *Laminaria flexicaulis* Le Jolis について報告を行っているが，我が国では，ただ，阿部氏 (1939) の *Laminaria japonica* Aresch. マコンブについての報告があるのみである。

それ故，その細胞学的研究の行われた種も少く，またこれらの観察に不充分な点もあるので，著者らはここに前のワカメ *Undaria pinnatifida* (Harv.) Sur. の引き続きとして，北海道に産するミツイシコンブ *Laminaria angustata* Kjellm. を

材料として，その游走子嚢の発生および，游走子形成の細胞学的研究を行ったので，その結果をまとめて発表する。

材 料 と 方 法

本研究に使用したミツイシコンブ *Laminaria angustata* Kjellm. は，1954 年 8 月 10 日 と 27 日の 2 回にわたり，北海道大学理学部附属室蘭海藻研究所で採集したものである。先ず，酢酸オルセインにより，游走子嚢の成熟程度を確かめ，熟した胞子葉を選び出し，これを著者らの前報のワカメの研究において用いた阿部氏液で固定した。固定時間は 24〜45 時間で，其の後，普通のパラフィン切片法により；2〜5 μ の切片を作り，10% 過酸化水素で 24〜40 時間漂白した後，大部分のプレパラートはハイデンハイン氏鉄明礬ヘマトキシリンで染色したが，幾らかのものは，サフラニンで染色し観察を行った。

観 察

（1）**游走子嚢の発生**——ミツイシコンブの胞子嚢群のできる部分は，通常，葉の裏面，すなわち，中帯部が突出している側であって，胞子嚢群は中帯部の両側に，黄褐色の 2 列の帯となって形成されるが，葉の両面に作られる事もある。

葉の組織は，表皮，皮層，髄の 3 層に区別されるが，成熟期に達すると，その表皮細胞に変化が生ずる。すなわち，1 層に整然と並んだ各表皮細胞は，1 核と数個の色素体を持った外側の長方形の細胞と，内側（基部）の小さい正方形の細胞と

に，葉の表面に平行な膜で，分たれるようになる
(Pl. XVI, Figs. A, B)。内側の小さい細胞を底部
細胞（basal cell）と呼び，外側の細胞は，これが側
糸のはじめで，其の後も，分裂する事はなく伸長
し，遂には単細胞側糸となるものである（Pl. XIV,
Fig. C）。この頃から，側糸の外膜は粘液により，
その厚さを増加し始め，側糸の頭部はたがいに密
接し，その外側にある粘液角皮　（mucilaginous
cuticle）と共に，後になって発生してくる游走子
嚢を保護する役目をもつ。

　間もなく，底部細胞はこのような側糸の間に突
起を出し，突起が或る程度，大きくなると，隔膜
により底部細胞から切り出され，これが游走子母
細胞となる（Pl. XIV, Figs. D, E）。この頃になる
と，側糸は甚しく伸長し，その頭部は大きくなる
が，基部は細くなって糸状となり，其の間に游走
子嚢が発生し成長する（Pl. XIV, Fig. F）。成熟
した側糸の頭部は，その外膜が非常に厚い為，棍
棒状を呈し，中央に一つの核を有し，核の周辺に
は幾つかの色素体が存在する（Pl. XIV, Fig. G）。

　(2) 游走子形成——底部細胞から切り出されて
間もない若い游走子嚢には，4〜7 個の色素体と，
静止核とが見られる。その核の中央には濃く染ま
る大きな仁が存在し，核内は非常に繊細な網目状
構造となっている（Pl. XV, Fig. 1）。やがて，核
はその大きさを増すと共に，網目状の構造は次第
に明瞭になってくる（Pl. XV, Fig. 2）。この頃，
一つの仁以外に約 8〜15% の割合で，仁に似てい
るが，仁よりやゝ小さい構造物が認められる（Pl.
XV, Fig. 3）。しかし，この構造物はヘマトキシリ
ンで染めても，またサフラニンで染めても，全く
仁と同様に染まり，仁との区別は困難である。間
もなく，濃く染色されるようになった染色糸はルー
プ（loops）を作り，徐々に核の一隅に集合す
る。これらの染色糸は常に仁と密接している（Pl.
XV, Fig. 4）。この染色糸のループは核周辺部に拡
がると共に，核腔内にも伸展し，典型的なオープ
ン・スピレム期（open spireme）となる（Pl. XV,
Figs. 5, 6）。この頃，游走子嚢は著しく成長し，游
走子嚢内には大きな液胞が観察される。その後，
染色糸は各所で肥厚し，ディアキネシス期に入る
が，染色糸が肥厚を始める頃から仁は消失し始め，
染色糸の肥厚が相当進んだ時には，仁は完全に消
失する（Pl. XV, Fig. 7）。ディアキネシス期には，

O, Y, V, II 等の形をした二価染色体が認められ，
その末期では 22 の染色体が数えられるが，核膜
はこの頃から不明瞭になってくる（Pl. XV, Figs.
8, 9）。

　次いで中期に入り，各二価染色体は規則正しく
赤道板に並び，その極面観では 22 の減数された
染色体が，側面観では紡錘体と，その両極に中心
体状の小粒が観察される（Pl. XV, Figs. 10, 11）。
しかし，この中心体状の小粒は，染色の弱いプレ
パラートでは観察されない事が多い。紡錘体の軸
の方向は，大部分，游走子嚢の長軸に平行か，ま
たは少し傾いているが，游走子嚢の長軸に直角な
場合も観察された。

　後期では，各二価染色体は一価染色体に分れて，
規則正しく両極に向って移動する（Pl. XV, Fig.
12）。このような後期像は他の分裂期のものに比
べて，非常に僅かしか観察されなかった。終期に
は，核膜も仁も再び現れ，2 娘核は最初互いに接
近しているが，間もなく離れてその間の距離を増
す（Pl. XV, Fig. 13）。2 娘核の移動の結果，二つ
の核の間には色素体はなく，細胞質も僅かで液胞
となっている場合が多い。2 娘核にも，仁以外に，
仁に似た構造物を持つものが認められた（Pl. XV,
Fig. 14）。

　次いで第二分裂に入り，その結果 4 核を生じる
が（Pl. XVI, Figs. 15, 16），第二分裂は 2 娘核が
互いに接近した位置のまゝ起る事もあり，この際
の二つの分裂像は非常に接近している。其の後，
引き続き 3 回の核分裂の結果，游走子嚢内には 32
の遊離核が形成されるが，これらの核分裂はすべ
て同時的に行われ，またそれらの分裂の方向は互
いに無関係である（Pl. XVI, Figs. 17, 18, 19,
20, 21）。

　核分裂の経過と共に，游走子嚢は大きくなり，
第五分裂終了後その頂端の膜は著しく肥厚し，游
走子嚢周辺に散在する色素体も，この頃数を増し，
一個ずつ核の周囲に移行し，その後，細胞質がそ
の周りに集合して游走子が作られる。かくして，
完熟した游走子嚢は長さ 75〜95 μ，幅 9〜12 μ の
大きさとなり，その中には，中央に一つの核と一
個の色素体を有する 32 の游走子が作られる（Pl.
XVI, Fig. 22）。

考　察

ミツイシコンブの游走子嚢発生についての観察結果は，他のコンブ目についての報告と同様，游走子嚢も側糸も表皮細胞から発生する故，これら二つの器官は全く相同なものである。

第一分裂前期に見られた，仁によく似た構造物は，第二分裂でも観察され，またサフラニンで染色を行っても全く仁と同様に染色されるので，*Dictyota* アミジグサ属（Williams, 1904），*Padina* ウミウチワ属（Carter, 1927）*Coccophora* スギモク属（田原，1929），*Hizikia* ヒジキ属（猪野・広江，1954a），*Sargassum* ホンダワラ属（岡部，1929；阿部，1933；猪野・広江，1954b；広江・猪野，1954）等で報告されている 'chromophilous spherule' ではなく，仁と同一物と思われる。

仁及び核膜の消失について，著者らが前に報告したワカメ *Undaria pinnatifida* (Harv.) Sur.（猪野・西林，1954）と比較すると，ワカメでは仁は中期に消失するが，本植物ではディアキネシス期に入る前に既に消失し，また核膜はワカメでは中期まで消失しないが，本植物ではディアキネシス末期に不明瞭となり，本植物の仁および核膜の消失はワカメに比して早く行われるようである。

ディアキネシス期に，色々な形をした二価染色体が観察されるところから，本植物の游走子嚢内における最初2回の核分裂は減数分裂である。後期の分裂像は非常に僅かしか観察されないので，後期は他の時期に比較して，非常に早く経過するものと思われる。

コンブ目に関する，これまでの細胞学的研究の結果，染色体数は，*Chorda Filum* では n=20（Kylin, 1918），*Egregia Menziesii* は n=8, 2n=16（Myers, 1928），*Pterygophora californica* は n=13, 2n=26（Mckay, 1933），*Eisenia arborea* は n=15, 2n=30（Hollenberg, 1939），*Laminaria japonica* は n=22（阿部，1939），*Macrocystis integrifolia* は 2n=32（Walker, 1952），*Laminaria Cloustoni* は 2n=22（Walker, unpub.），*Laminaria flexicaulis* は n=13, 2n=26（Magne, 1953），*Laminaria digitata* は n=8, 2n=16（Walker, 1954），*Undaria pinnatifida* は n=22（猪野・西林，1954）等と報告されているが，本種では，マコンブ *Laminaria japonica*，ワカメ *Undaria pinnatifida* と同様 n=22 である。

また，游走子嚢内に形成される游走子の数についても，*Chorda Filum* の 16（Kylin, 1918），*Alaria esculenta*（Sauvageau, 1918），*Laminaria saccharina*（Schreiber, 1930），*Pelagophycus porra*（Herbst and Johnstone, 1937），*Eisenia arborea*（Clare and Herbst, 1938），*Laminaria japonica*（阿部，1939）および *Undaria pinnatifida*（猪野・西林，1954）の 32，*Pterygophora californica*（Mckay, 1933）および *Eisenia arborea*（Hollenberg, 1939）の 32 時々 64，*Saccorhiza bulbosa*（Sauvageau, 1918）の 128 等種々報告されているが，本種では常に 32 の游走子が形成される。

中心体については，マコンブ *Laminaria japonica*（阿部，1939）では第一分裂で紡錘体の1極にのみ，中心体状の小粒が観察されているが，本種ではワカメ *Undaria pinnatifida*（猪野・西林，1954）と同様，第一分裂で紡錘体の両極に中心体状の小粒を認める事が出来た。しかし，*Pterygophora californica*（Mckay, 1933）で報告されているような，中心体の周囲に存在する濃密な細胞質は観察する事ができなかった。

要　約

1，游走子嚢および側糸の両器官共に，胞子葉の表皮細胞から発生する。

2，游走子嚢内における最初2回の核分裂は減数分裂であり，其の後，引きつゞいて3回の核分裂が行われて 32 の遊離核が形成され，その結果，32 の游走子が作られる。

3，本植物の染色体数は n=22 である。

4，第一分裂中期の紡錘体の両極には，それぞれ中心体状の小粒が認められた。

Summary

1.　Both the zoosporangia and the paraphyses originate from the superficial cells of the sporophylls.

2.　The first and the second nuclear divisions in the zoosporangium are meiosis, and then three mitoses take place successively to form 32 free nuclei. Consequently 32 haploid zoospores are preserved in a zoosporangium.

3.　The haploid number of chromosomes in *Laminaria angustata* is 22.

4.　In the metaphase of the first division, the centrosome-like small bodies are recognized at both poles of the spindle.

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Explanation of Plates

Plate XIV

Development of zoosporangia of *Laminaria angustata* Kjellm. All figures were drawn with the aid of an Abbe's drawing apparatus. All megnifications are ×900.

Fig. A.　Section of lamina in sterile portion: one layered meristoderm is followed internally by small cells of cortex.

Fig. B.　Transverse division of the meristoderm to form the basal cell and the future paraphysis.

Fig. C.　Growth of the paraphyses.

Fig. D.　Projection of the basal cell between the paraphyses.

Fig. E.　Transverse division of the basal cell to form the zoospore-mother-cell.

Figs. F, G.　Further stages of development: paraphyses and zoosporangia grow up and the

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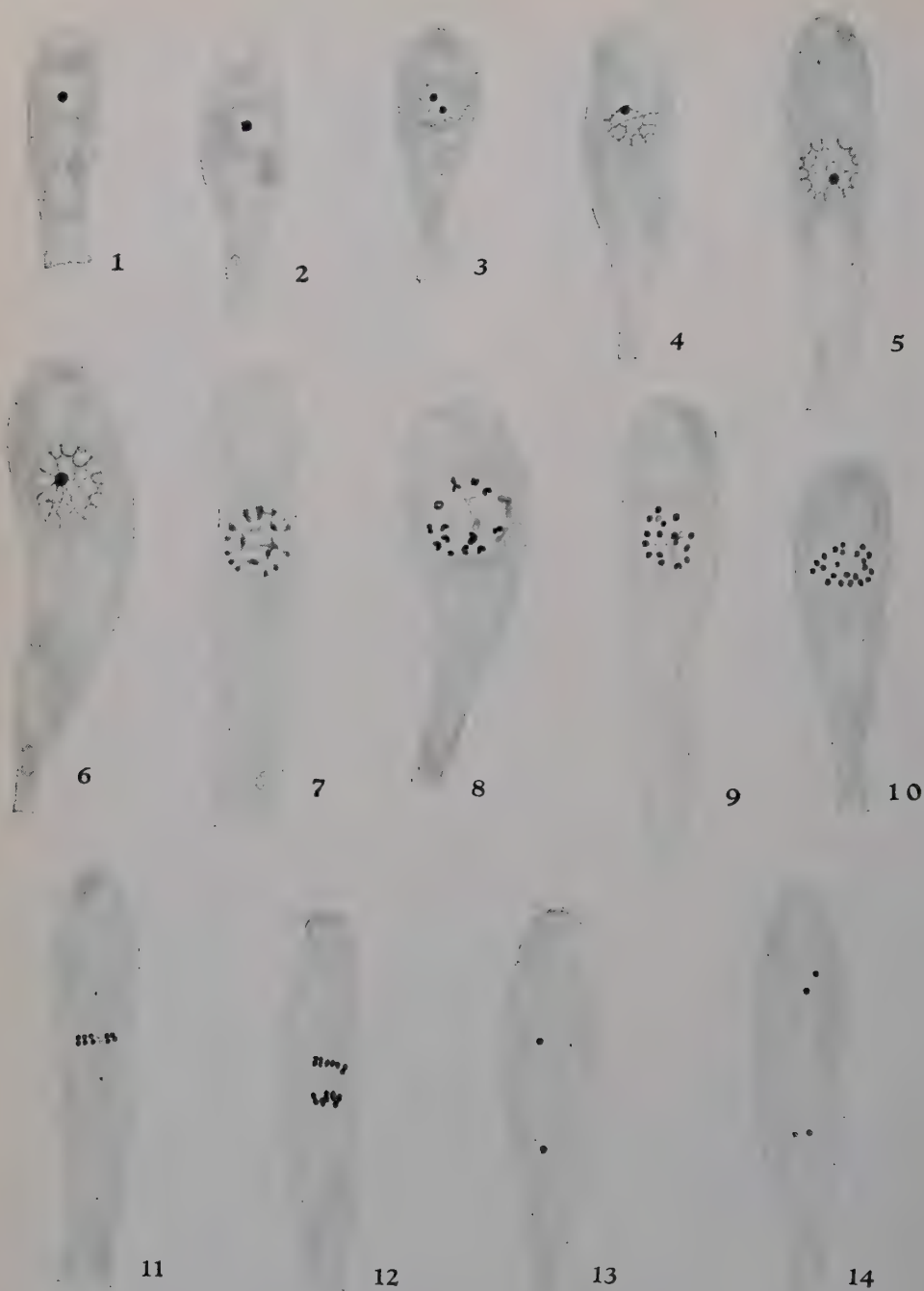
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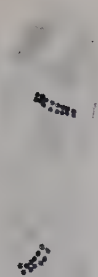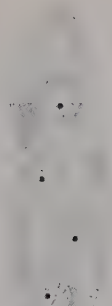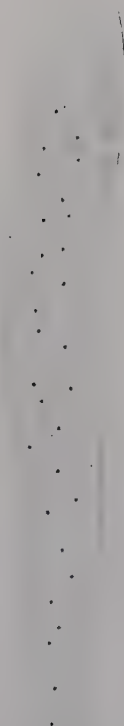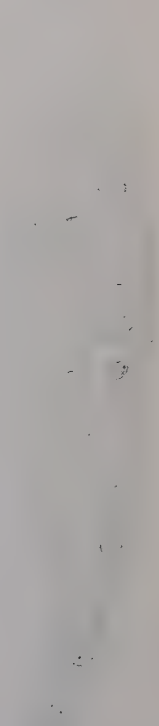
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wall at the apex of the paraphyses becomes much thickened.

Plates XV and XVI

Formation of zoospores in zoosporangia of *Laminaria angustata* Kjellm. All figures were drawn with the aid of an Abbe's drawing apparatus. All magnifications are ×2100.

Plate XV

Fig. 1.   Resting stage.

Fig. 2.   Later stage with reticular structure.

Fig. 3.   The same stage, showing two nucleoli in the nuclear cavity.

Figs. 4, 5.   Synapsis stages, showing chromonemata aggregated at one side or the whole periphery of the nuclear cavity.

Fig. 6.   Open spireme stage.

Fig. 7.   Early diakinesis.

Fig. 8.   Diakinesis, showing bivalent chromosomes with various forms.

Fig. 9.   Late diakinesis.

Fig. 10.   Polar view of the metaphase.

Fig. 11.   Side view of the same stage, showing the centrosome-like small bodies at the both poles of the spindle.

Fig. 12.   Anaphase.

Fig. 13.   2 daughter nuclei.

Fig. 14.   The same stage, showing two nucleoli in each nucleus.

Plate XVI

Fig. 15.   Metaphase of the second meiotic division.

Fig. 16.   4 nucleate stage.

Fig. 17.   Metaphase of the third nuclear division.

Fig. 18.   8 nucleate stage.

Fig. 19.   16 nucleate stage.

Fig. 20.   Metaphase of the fifth nuclear division.

Fig. 21.   32 nucleate stage.

Fig. 22.   Zoospores formed completely in a zoosporangium.

スギナの胞子の潜在極性

中 沢 信 午*

Singo NAKAZAWA*: The Latent Polarity in *Equisetum* Spores

卵や胞子の極性軸が外界条件の勾配によって支配されるという例は多く知られている[1, 3, 4, 5, 6, 8, 10, 11, 12, 13]。しかし，その場合に外界の影響で極性が新しくつくられるのか，それともはじめから潜在的に準備されていた極性の軸がその方向を変更されるにすぎないのか，という問題はなお残されている。もちろん，それぞれの場合によって同一ではないであろう。スギナについてはこの点に関して次のところまで研究されている。*Equisetum limosum* および *E. variegatum* について Stahl[10] の古典的な実験，これにつづく *E. limosum* と *E. palustre* についての Buchtein[1]，*E. arvense, E. limosum,* および *E. silvaticum* についての Nienburg[8]，また特に *E. arvense* についての Nakazawa[6] のくわしい実験によると，スギナの胞子は水中でたやすく発芽するが，そのさい極性軸の方向は定向的照射によって決定され，光原に遠い方の側に仮根極を生ずる。そして，Nakazawa[6] の実験によって，胞子を超遠心分離して内容物を分層してみても，極性軸はそれと関係なく決定されることから，極性の決定因子は超遠心によっても分層されない皮部細胞質にあることが推定された。しかし，その皮部の分化はいつ決定されるのかという点についてはなお未解決である。*E. arvense* では暗黒または散光のように一方からの照射のないところでも胞子の極性は決定される（Nakazawa[6]）のであるから，光は単に前からあった潜在的極性の軸の方向を変えるのみであろうと考えられるが，しかしそのような潜在極性がはたしてあるか否かはまだ決定的に知られていない。そこで筆者は外界条件を完全に均一にし，勾配を全く消して，その中で胞子を発芽させてもなお極性の分化が生じてくるとすれば，胞子は自身の内にはじめから潜在的に極性をもっていたと考えてよいと思い，そのような実験を行ったので，その結果を報告する。同様の実験は別に褐藻類のスギモク（*Coccophora Langsdorfii*）の卵についても行った結果，潜在極性があることがたしかめられている[7]。

実 験 と 観 察

材料は 1955 年 4 月に仙台で採集した スギナ（*Equisetum arvense* L.）の胞子で，実験は山形大学で行われた。実験方法はカエルの卵について行った Kathariner[2] の方法にならい，胞子を水道水にまいてからこれを二つに区分し，一方は径 10 cm のペトリ皿に水と共にいれて蓋をし，散光の下においた。この場合に胞子は重力はもちろん光や温度，pH などについても多少ともむらのある条件におかれている。ところが他方は径 3 cm，長さ 20 cm の試験管にいれ，水の深さ 10 cm とし，底にしかけたガラス管の口からポンプで気泡を出して水をかきまわし，こうして水に浮んだ胞子はたえず不規則に廻転し，すべての外界条件を均一にしながら発生させた。この胞子はときどきピペットで吸いとってしらべられた。観察にあたっては水面で気泡が破裂して，その飛沫と共にはね上げられ，上部のガラス壁に附着した胞子を混じえないように注意した。こうして静水中で発芽した胞子と不規則にうごかされて発芽した胞子とについて，形態的分化と生理的分化とを比較し，両条件で発芽に伴う分化の上に差異のないことを

* 山形大学生物学教室　Biology Department, Yamagata University, Yamagata, Japan.

証明した。生理的分化については生体染色によっ
て，前の実験で知られた特異な差次染色がどうあ
らわれるかを標準にしらべた。色素はサフラニン，
メチレンブルー，ブリリアント緑，ヤヌス緑，ナ
イル青などを用いた。染色液はすべて中性にした
水道水でつくり，1/100〜5/1000% とした。

　実験結果は表 1 および 2 に示した通りであっ
た。つまり胞子は光その他すべての外界条件に勾
配のない状態でも通常とひとしく発芽し，しかも
その条件で 36 時間以上も生長をつづけることが
できる。発芽の順序は，まず胞子が吸水してふく
らんでくると，やがて葉緑体が一方の半球に集合
し，胞子は分裂して 2 細胞となり，一方は大形で
葉緑体を多量にふくみ，他の一方は小形で葉緑体
をほとんどふくまず，後者はしだいに突出して第
一次の仮根をつくり，前者はさらに分裂および生
長して前葉体となる。水中で胞子が不規則に廻転

しながらも，仮根はしだいにのびて，ついには胞
子の直径が約 60μ に対して仮根の長さは 300μ 以
上にもおよぶ。それからは仮根がたがいにからみ
合って胞子は自由に廻転できず，綿のように一塊
となり，実験ができなくなる。生体染色すると，
各色素でいずれも仮根細胞だけが特に染めわけら
れる（図 1）。

　水に浮游する胞子の数をずっと少くして実験を
つづけると，仮根がからみ合うことなしに，なお
不規則に廻転させることができる。そうすると仮
根の生長は 300μ くらいで止まり，つぎには前葉
体となる方の葉緑体に富んだ細胞に分裂がおこ
る。そのさい，細胞膜ができる方向は A 型と B 型
との 2 種に分けられる。A 型は長軸と直角に細胞
膜をつくり，その結果，仮根の上に 2 つの前葉
体細胞がつみ重ねられたようになる（図 1 c, d；
図 2 a, c）。B 型は長軸に平行して細胞膜を生じ

Table 1.　Ratio in per cent of rhizoid formation of *Equisetum* spores,
in still water and being stirred irregularly, at 23 °C.

Condition	Time from the beginning of the experiment (hr.)				
	0	6	12	18	24
Still	0	0	44	86	93
Sirred irregularly	0	0	53	80	97

Table 2.　Growth of rhizoid of *Equisetum* spores placed in still water
and being stirred irregularly.　S=maximum diameter in
transverse, L=length of the longitudinal axis in μ, at 23 °C.

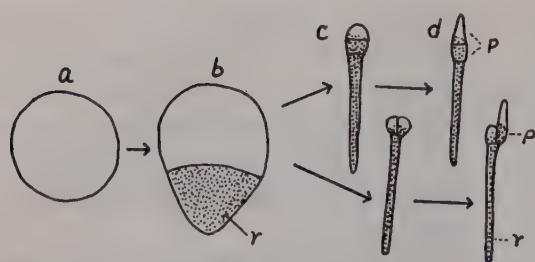| Condition	Time from the beginning of experiment (hr.)								
	0			24			36		
	S	L	L/S	S	L	L/S	S	L	L/S
Still	48	48	1.0	53	72	1.3	60	375	6.2
	//	//	//	63	73	1.1	//	300	5.0
	//	//	//	49	67	1.3	57	312	5.4
	//	//	//	58	69	1.1	65	360	5.5
	46	46	//	55	67	1.2	63	320	5.0
Stirred irregularly	48	48	1.0	56	78	1.3	65	320	4.9
	//	//	//	50	70	1.4	60	310	5.1
	//	//	//	//	76	1.5	//	385	6.4
	//	//	//	54	78	1.4	//	350	5.8
	49	49	//	50	69	1.3	65	360	5.5

Fig. 1.　Schematic illustration of A-type (upper course) and B-type (lower course) in germination of *Equisetum* spores.　a) Fresh spore,　b) the first cleavage,　c) the second cleavage in the prothallium cell,　d) growth of a prothallium cell;　p, prothallium cells;　r, rhizoid.　Dotting indicates the staining with brilliant green.

Fig. 2.　A-type (a, c) and B-type (b, d) in the germination of *Equisetum* spores. p, apex of the prothallium cells;　r, rhizoid.

その結果仮根の上に 2 つの前葉体細胞が平行に並べられたようになる（図 1 c, d; 図 2 b, d）。いずれの型でも前葉体になるべき 2 細胞のうちの一方はしばらく休止し，他方は直ちにまた生長をつづける。ところがこの場合に，A 型では頂端の細胞が頂きの方向に伸長する（図 1 d, 2 c）ように運命づけられているが，B 型では 2 つの細胞のうちの一方だけが機会的にえらばれて生長する（図 1 d; 2 d）。A と B の比は 1 : 1 にあらわれる。A 型か B 型かは Sachs[9] の法則にしたがい，第一次の分裂で生じた前葉体となるべき細胞が長軸の方向に長いときには A 型となり，横に長いときには B 型となる。この段階を生体染色によってしらべると，仮根が選択的に染められることは前段階と同じであるが，こんどはその外に，前葉体細胞

にも染色がおこる。しかしその染まり方は勾配をなし，基部からしだいに頂部に向って染まってゆき，頂端部は染められないでのこる。この事実は，これらの色素に対する透過性が基部ほど大きく，頂部ほど小さく，勾配をなしていることを示す。

以上の観察は，胞子が非水の中で発芽した場合についても，また不規則に廻転しながら発芽した場合についても，全く同一であった。

考　察

実験によって，スギナの胞子は外界条件に勾配がなくても発芽し，生長することが可能なことがわかった。つまり胞子の極性は外界の不均一性から影響されて生ずるものでなく，潜在的に内部に準備されてあり，外界が均一なときには自律的にその固有の軸にしたがって発生をすすめる。そのような潜在極性は，前回の実験[6] で明かにしたように，暗黒または散光下では仮根形成が弾糸の附着点と反対の側でおこりやすいことから，弾糸附着点をふくむ軸が潜在極性の軸だと思われる。なお発芽について，仮根の伸長や前葉体細胞の生長と分裂とも外界の勾配なしで行われる。従ってスギナの胞子では，少くともある段階までは形態分化はもっぱら潜在極性のプランに従って進行しうるものである。従ってまた Stahl[10] その他の実験のように，光の方向が極性軸を支配するのは潜在極性の軸を光が変更するにすぎないのであろう。それでは，この潜在極性の本質は何であり，それはどうして生ずるのであろうか。この問題はなお未解決であるが，褐藻類の卵についての筆者の他の実験[7]から考えてみると，潜在極性とは，おそらく原形質皮部の透過性が部分的に異る，言いかえると透過性勾配なのではあるまいか。スギナの胞子で 2 細胞期に仮根部がよりよく生体染色をうけ，また前葉体の細胞では基部ほど色素に対する透過性が大きいという事実は，この考えをうらがきしているようである。

Summary

Spores of *Equisetum arvense* L. were germinated in water, being rotated irregularly with air bubbles. As a result:—(1) It was revealed that the polarity axis was determined autonomically without the presence of a gradient of the external conditions. (2) The mode of germination was the same as that placed in the still water, both in morphological and in physiological characters. (3) A latent polarity, originally prepared in the spore cell, must be considered. Therefore, when the actual polarity is determined in the germination with the external condition, such as the unilateral illumination, it seems to be a modification of the latent polarity axis, but not a *de novo* formation of the polarity.

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ま ぎ ら わ し い 生 活 型

植物の生活型に関する二三の問題点 II*

沼 田 　 真**・浅 野 貞 夫***

Makoto NUMATA** and Sadao ASANO***: Ambiguous Types of Life-forms*
(Some Considerations Concerning the Biological Types of Plants II)

目 的 と 方 法

第 1 報[1]においてあげた植物の生活型に関するいくつかの問題点のうち，とくに生活型の判定上

問題となる場合について報告したい。一番問題になるのは，地表面を中心としたわずかの範囲に休眠芽が分布する場合であり，したがつて半地中植物，地表植物，地中植物の一部がこれに該当する。従来わが国で発表された報告のなかにも，判定の誤りと思われるものが非常に多い。われわれ自身，多くの個体を検討してみて，その判定が意外に困難な場合のあることを体験した。とくに，一時期，一個体から判定することの困難な場合にはしばし

ば出あう。本報においては，これら従来，不明確
な扱いをうけている植物として典型的なものにつ
いて，われわれの検討した結果の一部を報告する。

　従来の報告で同じ種が異った生活型に分類され
ている場合，それがそれぞれの植物気候の反映と
していずれも正しい場合もあるが，判定の誤りで
あることもしばしばである。というのは，休眠芽
の位置が外的条件によって変動しやすい場合とそ
うでない場合があるからである。たとえば，ヒメ
ジョオンは東京附近ではふつう生活期間がほぼ一
年にわたる越年生草本で Th にはいるが，所によ
り，さらにその翌年地上部のほとんど枯れた根際
から芽を出して生活期間の短い半地中植物とみな
される場合がある。しかしこのような変動をしめ
すかどうかは種によってちがうので，判定がまち
まちである場合に，その正否は個々の種について
検討されなければならない。そのような場合の厳
密な判定が，従来，種の同定のごときに比して少
しくおろそかにされていたように思われる。その
ような誤りをさけるためには，地上部から直観的
に生活型を判定しないこと，地下部（とくに ra-
dicoid type[2]）をよく検討すること，できるだけ
多数個体にあたること，などが大切であろう。

　本報における標本採集の場所としては，おもに
千葉県南部（とくに安房郡）にもとめ，十分はっ
きりしないものは長期の観察を行い，一部は移植

して観察した。個体数は少くとも 10 個体以上を
えらんで検討するようにつとめた。判定に使用し
た資料は腊葉標本として保存してある。

資 料 と 論 議

　図 A） フユイチゴ *Rubus buergerii*. ミヤマ
フユイチゴ *Rubus hakonensis* のような常緑のつ
る性灌木であるが，比較的短期間で枯れることが
多い。図からわかるように，地上茎基部の越冬芽
は半地中植物型であるが，フユイチゴ（清澄山 11
月所見）では，この越冬芽の表面はほとんど常に
落葉層におおわれ（他の雑草中にうもれることも
ある），radicoid の越冬芽の面からは地中植物型
のようにみえることさえある。（もっともその落
葉は，ふつうシイ，サカキなどの落葉をバラバラ
とかぶった形になっていることが多い。）

　ここで s' は前年の，s は新しい地上茎である。
radicoid の越冬芽（A の右図）のうち，地中の b'
は小さくて結局は駄目になり，地表面に顔を出し
た b の芽が大きくて赤味があり元気よく，これだ
けが育つのである。

　ところで生活型の判定には，いうまでもなく最
高の休眠芽の位置が基準になるのであるから，フ
ユイチゴのようなつる性灌木では，地上茎の腋芽
の位置が当然問題になる。たしかに腋芽が伸長し
て新条になり（A の左図，b），旧い枝についた葉
はやがて枯れる。ところがこの腋芽は，
多くの場合，つる性の茎が地についてそ
こに根をおろし，そこの腋芽が伸長して
新条となる。

　以上のような理由から，常緑のつる性
灌木とはいいながら，少しくまぎらわし
い形の地表植物（その細別からいえば
いわゆる Spaliersträucher すなわち
Chamaephyta velantia と匍匐性草本
Chamaephyta reptantia の中間の性質
をもつもの）[3] に属するのがこのタイプ
である。

　図 B） radicoid の冬芽のつき方は地
中植物型であることが多いが（B の右
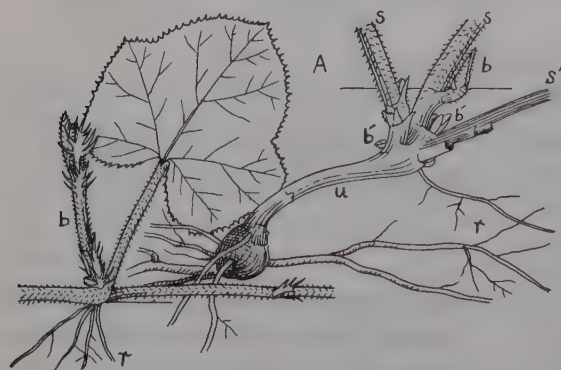図．実線が土壌表面），斜面に生育する
場合，表土が流亡して（点線は流亡後の
土壌表面），半地中植物型，ないしは地表

Fig. A.　Aerial shoots (left) and underground part
(right) of *Rubus buergerii* at Mt. Kiyosumi in
November.　s: aerial stems of the current season,
s': aerial stems of the former season, b: peren-
nating buds to grow in the following season, b':
buds to wither shortly, u: underground stems, r:
roots.
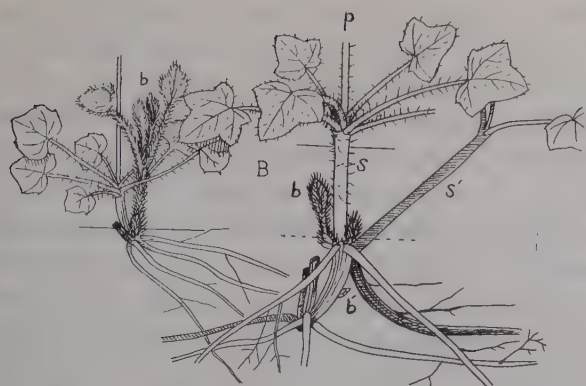
Fig. B. *Ainsliaea apiculata* at Mt. Kiyosumi in January (right) and April (left). When growing on a slope, the soil surface (solid line) often lowers by the erosion (broken line: a new surface of the soil). p: peduncle.

植物型にさえなることがある。そのような状態で生活が維持されるかぎりは、それぞれに半地中植物ないし地表植物と判定してもよいであろうが、左図のようなロゼット型に近い半地中植物とみるべき場合が多い。今年の地上茎としては b（左および右図）の芽が伸びるのであり、b' のような深い芽は駄目になってしまうことが多い。図は、キッコウハグマ *Ainsliaea apiculata* の清澄山における 1 月（右）と 4 月（左）の状態である。常緑草本ではあるが、茎の頂端から花梗（p）が一度でるだけで、二度でることもなく、その頂端に越冬芽が形成されることはない。昨年の葉は今年の秋になるとほとんど枯れるが、もっと早い時期に枯れる葉もある。要するに、多数個体のさまざまな場合の検討から、一般に半地中植物とみなすの

が妥当である[6]と考えられる。

　図 C) 越冬芽の位置は、地中、地表、地上といろいろなところにつくが、このうち前年の地上茎（s）についている越冬芽 b' は、一般にその後の発育がわるく、やがて枯れてしまう。ある時期の生きた越冬芽の位置ということだけならば、当然、地表植物になるが、このような、やがて枯れてしまう活動的でない芽の位置で生活型を判定するのは適当でないと思う。b の芽が今年の地上茎になる。地中植物と見なされる場合もあるが、代表的には半地中植物と判定される。イノコズチ *Achyranthes japonica*、ヤナギイノコズチ *A. longifolia* のごときがこの例である。図は、清澄山で 2 月の状態。

　一年生植物とされている場合があるが[4]、これは明らかに誤りであると思われる。堀川・佐藤（1936）は、イノコズチを、越冬芽が地上茎の基部に存する protohemicryptophyte の例としてい

Fig. D. *Plectranthus inflexus* at Amatsu-machi in November.

るが[5]、一般にはそのような判定でよいであろう。

　図 D) これは、地表に顔をだす芽の先端部によってしばしば半地中植物と見誤られるが[6]、芽の位置は明らかに地中であって、地中植物に属する。この図のように地下茎の肥大した部分が 2〜3 個ついていることが多いが、肥大部が地表に近いとき、とくに半地中植物と誤認しやすい。肥大部が 1 個のときは、ふつう肥大が大きく、またその位置も地表から離れて、明瞭な地中植物の型を
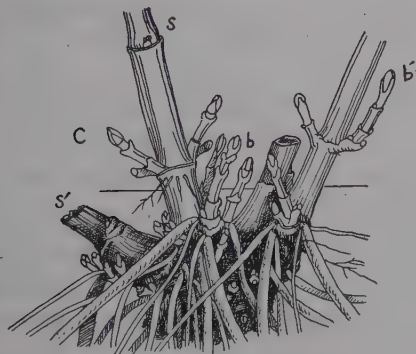
Fig. C. *Achyranthes japonica* at Mt. Kiyosumi in February.

しめす。図はヤマハッカ *Plectranthus inflexus*, 天津町の山道でえられた資料で，11 月の状態である。
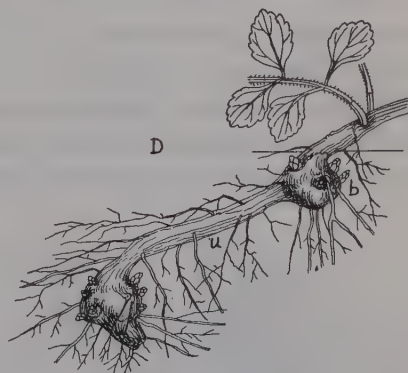
図 E) これも地中植物なのであるが，越冬芽が大きく，往々鱗葉をかぶった先端部が地表にあ
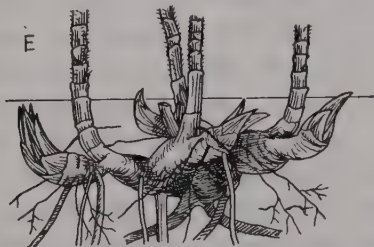
Fig. E.　*Agrimonia eupatoria* var. *pilosa* at Mt. Mineoka in November.

られるので，直観的に半地中植物と誤認されやすい。実際に地下部をほってたしかめないと間違える公算が大きい。

図はキンミズヒキ *Agrimonia eupatoria* var. *pilosa* の 11 月（峯岡山山道所見）のものであるが，冬の間はこのままの状態で，芽はほとんど大きくならない。キンミズヒキを半地中植物と記載している場合があるが[4]，誤りと考えられる。

図 F) 繁殖型からいうと R₃ 型の，いわゆる Horstpflanze すなわち Hemikryptophyta caespitosa で，半地中植物の一つの典型的な形ではあるが[6]，図の断面からわかるように，越冬
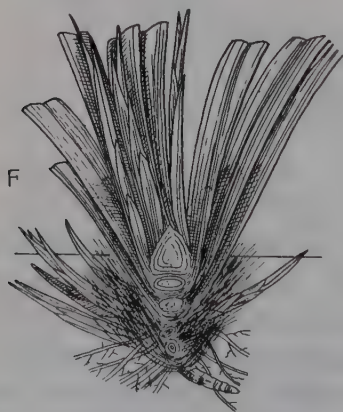
Fig F.　*Carex morrowii* at Mt. Kiyosumi in January.

芽は上下かなりの範囲に多く簇生する。われわれの経験によれば，このため，時に地表植物ないし地中植物と誤認される場合があった。カンスゲ *Carex morrowii* やミヤマカンスゲ *Carex multifolia* などがよい例である。図はカンスゲ，清澄山で 1 月の状態をしめす。

図 G) シュウブンソウ *Rhynchospermum verticillatum* のような例であるが，11 月頃（図の状態）すでに越冬芽（b）が開舒しているため地表植物と誤認されやすい。また半地中植物とみなされる場合もある[6]。正しくは地中植物に属する多年生草本である。

一方，牧野 (1940) は越年性草本としているが[7]，厳密には誤りであろう。生活様式からみると，ヒメジョオンのような partial rosette のタイプに類似したところがある。前年の地上茎（s'）は枯れたまま残存し，今年の地上茎（s）の基部から翌年の地上部がロゼット型に早く開舒し，この形で越年する形をとる。
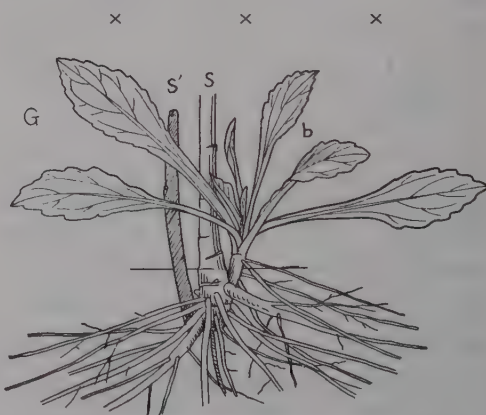
Fig. G.　*Rhynchospermum verticillatum* at Mt. Kiyosumi in November.

判定を誤りやすい生活型が以上でつきるわけではないが，ここにはその典型的なものをあげた。われわれは今までの数少い生活型目録の中に多くの誤りを見いだしているが，それらの誤りには共通したいくつかの原因がある。ここにあげた数箇の場合は，地表面を中心としたわずかの範囲に休眠芽が分布する場合についての基本的な事例と考えられる。

Summary

1. Among several important problems concerning the field survey and statistics of biological types of plants, some cases difficult to judge a life-form to which a plant belongs are discussed in this report. Heretofore the identification of life-forms seems to have been done without much consideration as compared with that of species. To avoid such misjudgements, it will be most important that many individuals are compared and especially the underground parts of plants are examined.

2. In this report, ambiguous types of life-forms particularly concerning hemicryptophytes, chamaephytes, and geophytes as shown in Fig. A~G are discussed.

A) This is one of evergreen lianas, not climbing, and a creeping chamaephyte. The highest perennating buds are lateral buds (b in the left figure) on the creeping stem from which some adventitious roots grow (Fig. A).

B) An evergreen herb as a rosette-like hemicryptophyte. No terminal perennating bud is formed (Fig. B).

C) The highest perennating buds (b') wither in a short time. This seems to be a protohemicryptophyte as a rule (Fig. C).

D) A geophyte with an obliquely ascending rhizome. When a tuberous enlargement is near to the soil surface, it is apt to be misjudged as a hemicryptophyte (Fig. D).

E) A rhizome geophyte which is apt to be misjudged as a hemicryptophyte for their large scaly buds often appearing on the soil surface (Fig. E).

F) A tussock-like rhizome hemicryptophyte which is apt to be misjudged as a geophyte or chamaephyte (Fig. F).

G) A geophytic perennial herb. As the perennating bud on the basal portion of an aerial stem grows already in November to a rosette-like plant, it is apt to be mis judged as a hemicryptophyte or chamaephyte (Fig. G).

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Chrysanthemum japonense Nakai に於ける
倍数性と地理的分布

下斗米直昌*・田中隆莊*・益森靜生*・石黒道也*

N. Shimotomai*, R. Tanaka*, S. Masumori* und M. Ishiguro*: Über die Polyploidie
und geographische Verbreitung bei *Chrysanthemum japonense* Nakai.

緒　言

　一種類の生物が天然に広く分布している場合，分布の区域が異なり，異なる環境に生育すれば分化を来すことは種々の生物に於いて確められつゝある。われわれが研究して来ているキク属及びその近縁属植物に於いても，夫々の全分布区域を調べると多かれ少なかれ分化を示すものが多い。又多形性を示し，分類学上変種の存在が記されているものもある。それ故にキク属及びその近縁属の各種につき夫々の分布区域に於ける分化を形態学的細胞学的及び遺伝学的に詳しく研究することが望まれるのであって，かゝる研究によってこれらの属に於ける種の生成と類縁が明らかにされるであろう。われわれの研究室に於いてはこれらの属のいくつかの種に於いてこの種の研究を行い成果を得つゝあるが，本報告にはノジギクに関する研究結果を示す。

　ノジギク (*Chrysanthemum japonense* Nakai) はキク属の本邦産の１野生種であって，四国，九州の沿岸及び瀬戸内海の沿岸の処々及び島嶼に生育しているものであるが，なお奄美大島など西南諸島にも分布している。(下斗米 1933, 北村 1940)。本種に於いては形態上の変異性が認められ，又変種も記載されている。北村 (1940) は本種に３変種を記載して居り，同氏の見解は本種の多形性の一端を示すものとして注目に値するものではあるが，その観察では生態型と因子型との区別が明ら

* 広島大学理学部植物学教室　Botanisches
Institut, Hiroshima Universität, Hiroshima,
Japan.

かでなく，本種に於ける多形性の本質を明らかにするまでに至っていない。

　筆者等はノジギクを多くの生育の場所より採集し，これについて形態学的比較研究をすると共に，その染色体数を観察し，なお遺伝学的研究をも行いつゝあるが，本種には 2n=54, 72, 90 の種内倍数性があること，及び倍数性と地理的分布の間に関係があることが判ったので，本報告にはこれらの結果について概略を記す。

材　料　と　方　法

　最近数年間われわれはノジギクの多くの生育の場所に行き形態学的観察をすると共に分布を調べ，多くの株を採集し，これを園に植えその一部は植木鉢に培養した。これらの株につき形態学的比較研究を行うと共に，その染色体数を調べた。染色体数は根端細胞に於いて算定したが，根端処理は Tjio 及び Levan (1950) の方法によった。即ち 0.002 M のオキシキノリンを 15〜18°C に保ち，これに根端を 90〜120 分入れ，次に酢酸の 45% 水溶液で数分間固定し，60°C の 1N の塩酸に約 15 秒間入れて加水分解を起さしめ，酢酸オルセイン液に移して染め，最後にスライド上に移してカバーグラスをかけて押しつぶすのである。押しつぶしには最初指で，次いで加圧器を用いて圧力を加えて染色体ができるだけ一平面に並ぶようにする。

研　究　結　果

　1.　6 倍型 (2n=54)。四国，九州及び瀬戸内海沿岸及び島嶼産ノジギクは 2n=54 即ち 6 倍型で

あることは従来の研究で知られている（下斗米1933, 1935）。戦後われわれは再びノジギクをまず上記の範囲内の多くの生育の場所より採集したが，採集の場所は次の通りである。播摩国：大塩。安芸国：向宇品，向灘，能美島，江田島，広。周防国：室積，室津〜阿月，上関，祝島，長島。伊予国：宇和島，大井，俵津。土佐国：高知龍王岬（桂浜），新宇佐白鼻，足摺岬。日向国：青島。薩摩国：谷山，山川。

　これらの地より採集された株の中で現在までに染色体数の調べられたものはすべて 2n=54 である。以前の研究及びこの報告の研究結果から明らかなように，四国，九州，瀬戸内海沿岸及び島嶼産ノジギクは 2n=54 即ち 6 倍型である。

　北村はノジギクに 3 変種即ち var. debilis Kita-

Fig. 1. 　種子島及び屋久島に於ける *Chrysanthemum japonense* の分布。斜線はその集団の存在を示す。〇印は確められた hexaploid, ●印は確められた octoploid.

mura セトノジギク（瀬戸内海沿岸産），var. *ashizuriense* Kitamura （足摺岬産），及び var. *crassum* Kitamura オオシマノジギク（奄美大島産）を記載している。これらの 3 変種中 var. *crassum* は後に記すように 2n=90 即ち 10 倍型であるが，var. *debilis* 及び var. *ashizuriense* は下に記すように共に 2n=54 で四国，九州産ノジギクと染色体数に於ける相違はない。

　瀬戸内海の沿岸例えば大塩の海岸，向宇品の海

岸及びその附近に生えているノジギクには頭状花や葉の形に関して著しい変異が見られる。又葉の形質にはシマカンギクの形質の入っているものも発見される。例えば向宇品のノジギクには葉が薄く，鋸歯が鋭いものが多く，頭状花の形にも変異が著しい。かような事実は瀬戸内海に入って来ているノジギクによく見られる事実であつて，北村が var. *debilis* と呼んでいるのはこれに該当するものと思われる。かような形質を示すものの染色体数も殆んど皆 2n=54 であった。例えばわれわれは向宇品に於いて生育するノジギクについて，著しい変異を示すものをも含めて 22 株の染色体数を調べたが全部 2n=54 であつた。また足摺岬産ノジギクはわれわれも観察したが，一種の生態形で 2n=54 であって，染色体数には変化が来ていない。なお大塩産ノジギクも 2n=54 である。

　最近われわれは種子島に於けるノジギクの分布を調べ，その染色体数を研究した。この島に於けるノジギクの分布については東海岸の一部を除き殆んど全島の海岸につき調査した。大きな集団の見られるのは北端の御崎附近，東海岸の安納，庄司浦，熊野，西海岸の竹之川，牛野〜大川間及び最南端の門倉岬等である。これ等の地に於けるノジギクの生育は山と海岸との間の斜面，或は防風林と海岸の間等である。これらの地より採集したものにつき染色体数を調べたが，現在までに調べた 23 株中 21 株は 2n=54，残り 2 株は異数性で 2n=55, 2n=53 が各各 1 株ずつである。本島産ノジギクについては研究すべきことがなお残っているので詳しいことは後日に譲る。

2. 8 倍型（2n=72）。われわれは屋久島産ノジギクは 2n=72 即ち 8 倍体であることを確めた。

　この島に於けるノジギクの分布については，西海岸の原川，半山附近は未調査であるが，この部分を除いた本島の殆んど全周囲につき調べたが，ノジギクの集団を南部の中間，湯泊，平内，尾の間，原等の海岸附近に於いて認めた。また東北部即ち永田岬附近には本種の多数の集団がある。東より北に及ぶ海岸及びその附近にはノジギクが見

られなかったので，この間には本種が恐らく生育していないものと考えられる。

　なお本島産ノジギクについて特に指摘しなければならないのは，南部産と西北部産は，染色体数には差がないが，形態上には明らかに差があることである。即ち南部産は葉の形態及び葉の裏面の毛の少ないこと等で奄美大島産ノジギクに似ているが，西北部産特に永田岬産は葉形及び葉の裏面に白毛の極めて多いこと等でサツマノギク (*Chrysanthemum ornatum* Hemsley) によく似ているのである。

3.　10 倍型 (2n＝90)。奄美大島及び徳之島に於いてわれわれはノジギクを採集したが，これらの島のノジギクは北村が var. *crassum* と記載したものである。奄美大島に於いては西側の名音，朝仁，名瀬，円及び安木屋場に於いて採集したが，
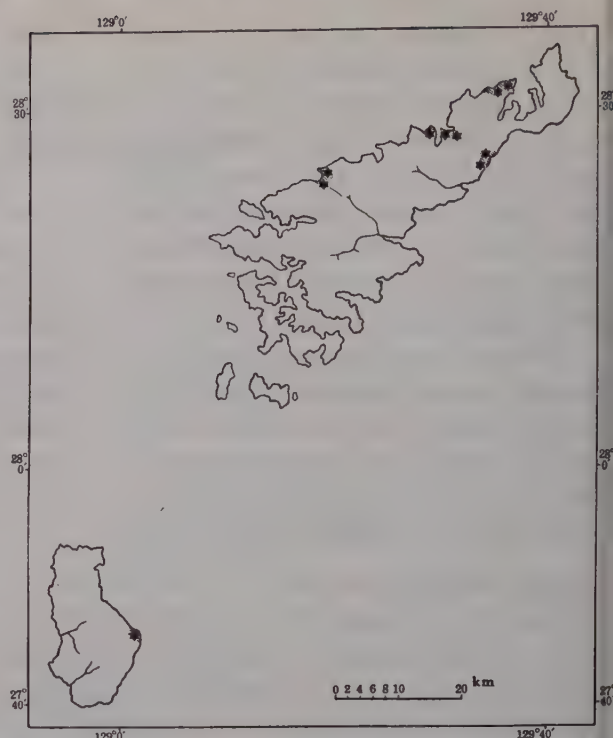

Fig. 2.　奄美大島及び徳之島に於ける *Chrysanthemum japonense* の分布。斜線はその集団の存在を示す。★印は確められた decaploid.

3

4

5
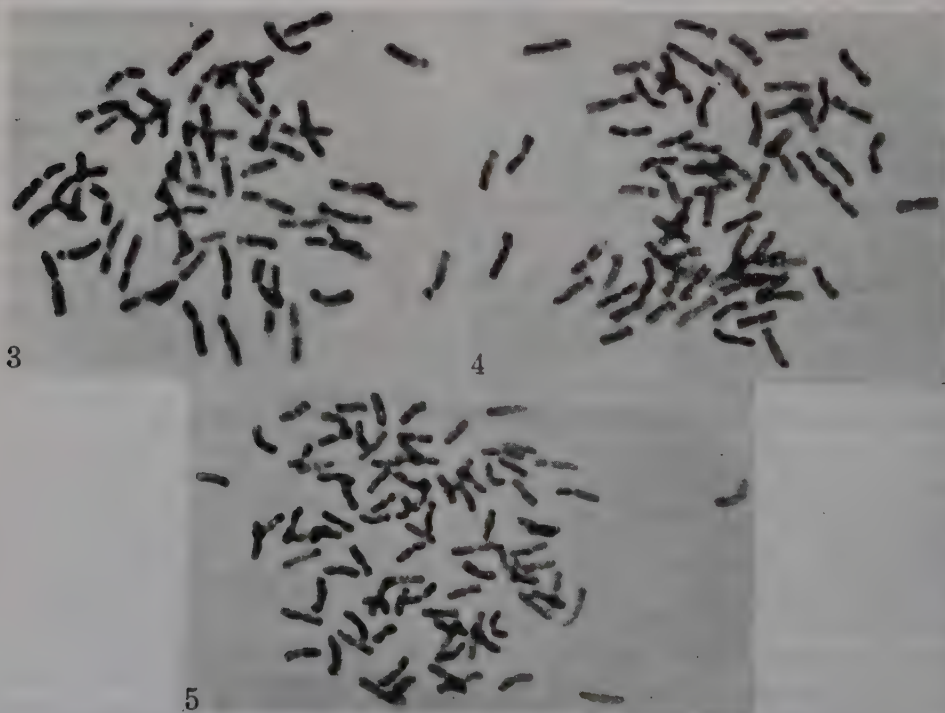
Fig. 3.　種子島産 *Chrysanthemum japonense* の hexaploid の根端細胞の染色体 (2n＝54)。×2250。
Fig. 4.　屋久島産 *Ch. japonense* の octoploid の根端細胞の染色体 (2n＝72)。×2250。
Fig. 5.　奄美大島産 *Ch. japonense* の decaploid の根端細胞の染色体 (2n＝90)。×2250。

これ等の地に於いてはノジギクは海に臨む斜面等に多い。東側では戸口に於いても採集したが、こゝにも多数生育している。徳之島に於いては神之嶺及び亀徳に於いて本種を採集した。

上記の諸地より採集したノジギクはその染色体数を算定するに、現在までに調べた範囲内では、すべて 2n＝90 である。即ち 10 倍体である。

われわれの奄美群島に於ける採集は上記の地に限られているが、大野昭好氏は本種を多くの地で採集している（個人的報告）。即ち奄美大島では北端及び東海岸の多くの地に本種を採集し、又加計呂麻島、請島、与路島、及び喜界島及び徳之島で本種の生育を確めている。即ちわれわれが奄美本島及び徳之島に於いて確め得た 10 倍型は大島の他の多くの個所及び附近の島々にも生育しているものと思われる。

奄美大島及び徳之島産ノジギクは他の地方産のノジギク即ち 6 倍型及び 8 倍型に比較して遙に大きい。かように形態の大きいのは染色体数の多いことに原因するものと思われる。又この 10 倍型は成長及び開花の時季等についても特異性を示すがこれについては後日報告するであろう。

論　議

ノジギクに於ける多形性についての従来の観察は 6 倍型に於ける変異である。即ち 6 倍型に於いては極めて稀れに異数性が出現するのであるが、染色体数の大きな変化はないのである（下斗米 1935）。即ち四国、九州、本州に於ける分布区域内に於いてはノジギクは染色体数 2n＝54 で安定しているのであって、変異の見られるのは多くは遺伝子に於ける変化でなければならない。又本属他種例えば Chrysanthemum indicum の 6 倍型即ちハマカンギクとの天然雑種より導かれたもの、或は分布の限界に於いてしばしば見られる僅か雑種性の混入しているものはその染色体の中に Ch. indicum の染色体の混入の結果とも考えられるが、しかしこれらの場合には染色体数には変化は起きていない。また足摺岬産の var. ashizuriense と記載された生態型も染色体数には変化はない。種子島産のノジギクに於いては染色体数は 2n＝54 でまれに異数性が出るのであるが、この地はノジギクの 6 倍型の南限であるから形態及び染色体数の変異については、他の地に比較して頻度が多

いかどうかについて目下研究中である。

次に屋久島のノジギクは 8 倍型であり、奄美大島及びその附近産のノジギクは 10 倍型であるから、染色体数の相違は地理的分布の差に関聯していることが判るのである。ここに明かなことは一の島に於いては 6 倍型、8 倍型、10 倍型のいずれか 1 つだけが存在し、2 の型がある場合が未だ見出されていない。種子島、屋久島、奄美大島の 3 島の附近にある島に於いては 2 つの型のノジギクが共に生えていることが或いは見出されるかも知れないが、もしかような場合が見出されるならば、これら 3 型の間の密接な関係が一層明かにされるであろう。かような場合が見出されない現在に於いても、この 3 型は形態上互に似ていること、又形態上の差違としては 8 倍型及び 10 倍型は 6 倍型より導かれた同質的な要素を多く有する点等から考えて、ノジギクの分布が南方に拡大すると共に 6 倍型より 8 倍型及び 10 倍型が生じたものと推定される。ノジギクに於いては 6 倍型が原型であって、その本来の生成の地は恐らく四国の海岸又は九州の東海岸であろうと思われるがその分布が南方に伸び屋久島、奄美大島等に至って分化したのである。

サツマノギクはノジギクに比較すれば、形態上似た点が多いが、茎太く、節間短く、葉厚く、頭状花が一層大きい等のために、ノジギクより染色体の増加によって生じた所謂巨大型とも見做し得るものである。その上その地理的分布は薩摩半島より羽島崎に至る間であって、ノジギクは鹿児島湾沿岸にも生えているから、ノジギクの分布区域に極めて近いのである。かような事実から考えて、サツマノギクはノジギクから導かれたものと推定されるのであるが、屋久島産ノジギクはサツマノギクと染色体数が同じく、その上その西北部に生えているものには形態にサツマノギクの形質を有すること等の事実は、上記の推定を一層有力ならしめるものである。即ちノジギクは南方に分布するにつれ分化して 8 倍型及び 10 倍型が生じ、8 倍型が分化してサツマノギクになったものと考えられる。かような見地よりすれば、Chrysanthemum japonense はそれ自身に於いて種内倍数性を形成しつゝ分化して復雑化して居り、またサツマノギクはこの分化に関聯して生じたに相違ない近縁のものであるから、これらを綜合して Chry-

santhemum japonense Complex と称し得るのである。Clausen, Keck 及び Hiesey (1940, 1945, 1948, 1951) はいろいろの種を分類学的，細胞学的及び生態学的に詳細に研究した結果 Species Complex の例をいくつかの種に於いて明らかにしているが，この報告に記したわれわれの研究結果は本邦産植物に於いても同様の現象が起っていることを示すのである。

摘　要

1. 筆者等はノジギク (*Chrysanthemum japonense*) を本州，四国，九州に於ける多くの場所より採集すると共に種子島，屋久島及び奄美群島に於いてその地理的分布を調べ，採集した株について形態学的及び細胞学的研究を行った結果，本種に6倍型 (2n=54)，8倍型 (2n=72)，及び 10 倍型 (2n=90) があることが明らかになった。

2. 四国，九州，瀬戸内海の沿岸及び島嶼産ノジギクは6倍型であることが従来の研究で判っていたが，筆者等はこれらの諸地方の多くの場所のノジギクの染色体を調べた結果，みな6倍型であることを確めた。又種子島産ノジギクは6倍型であること及びまれに異数性が存在することを確めた。

3. 屋久島のノジギクは8倍型であること及び西北部に生育するものはサツマノギクに形態が似ていることを知った。

4. 奄美大島及び徳之島産ノジギクは 10 倍型であることが明らかになった。

5. ノジギクは地理的分布と関聯して3型に分化し，その中の8倍型からサツマノギクが導かれたことが明らかになった。

Zusammenfassung

Die Verfasser haben *Chrysanthemum japonense* Nakai an verschiedenen Fundorten von Honshiu, Shikoku, Kiushiu und einigen Inseln des südwestlichen Meeres von Japan gesammelt und haben daran morphologische und cytologische Beobachtungen ausgeführt. Die Untersuchungen haben ergeben, dass es bei dieser Art die Polyploidie mit den Chromosomenzahlen 2n=54, 72, 90 gibt.

Die hexaploide Rasse kommt in Shikoku, Kiushiu und Honshiu sowie auch auf der Insel Tane-ga-shima vor. Die octoploide Rasse tritt auf der Insel Yaku-no-shima auf. Die Klone, die am nordwestlichen Teil dieser Insel wachsen, weisen in äusseren Merkmalen eine starke Ähnlichkeit mit *Chrysanthemum ornatum* Hemsley auf. Die decaploide Rasse befindet sich auf den Amami-Inseln.

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コウヤマキの葉の厚膜細胞の発達について*

北　村　玲　子**

Reiko KITAMURA**: Development of the foliar scereids
in *Sciadopitys verticillata* Sieb. et Zucc.*

　厚膜細胞については在来多くの報告があり，数百の属の植物の各組織に存在することが知られている。中でも葉肉部に存在するものが最も多く，ここに扱ったコウヤマキもまたこの一例である。これらの報告のうち古い年代のものは形態の記載に終っているが，近年に至りその発達段階を形態学的，組織学的，発生学的見地から追究することが盛に行われるようになってきた。最近では Sterling (1947), Rao (1949, 1951, 1952), Arzee (1953 a, b), 及び Foster (1955 a, b) の研究等がこの例である。

　ここに材料として用いたコウヤマキ *Sciadopitys verticillata* Sieb. et Zucc. の葉肉に観察される樹枝状厚膜細胞は，毬果植物中の稀有な例として古くから知られていたものである。一方この植物の葉は，4 月上旬葉が伸び始めてから成熟葉に至るまでに実に 100 日以上を費す緩慢な成長を行うものである。この研究は，葉の成長を日を追って詳細に観察するとともに，厚膜細胞の発生から次第に発達して行く段階を追究したものであるが，葉の成長と厚膜細胞発達の両者の時期的な関連性については，特に意を用いて論及した。

材 料 及 び 方 法

　東京大学理学部附属植物園内の 1 株のコウヤマキについて，新葉の開く時期を 3 月中旬から観察していたところ，4 月 7 日に到り鱗片葉が開き始めたので，この日に最初の採集を行い，以後 4 月 21 日までは 3 日おきに，7 月 21 日までは約 7 日おきに，何れも典型的な発育を行いつつある約 20 葉ずつを採集し，葉全体を F. A. A. で固定した。

　1.　この各段階の約 10 葉について，葉の全長にわたり 10 μ（若い段階）—30 μ（晩い段階）のパラフィン切片（横断，及び葉面に平行な縦断）を作り，ヘマトキシリンとサフラニンで染色した。又一方，8% HCl に葉身全体を浸し，5—10 分間加熱し，全細胞を解離し水洗した上，遠心分離器にかけて沈澱した細胞群を少量ずつピペットでスライドグラスに取上げ，サフラニンを混ぜたグリセリン・アルコールで封じた。

　2.　分布状態の観察を容易にするため，成熟葉全体を 1 モル KOH の中に 7 日間浸し 40°C に保ち透明化を試みたが，葉肉が厚いためなお不十分であったので，Fig. 2. C に示すように破線の位置でかみそりで縦に切目を入れ，葉を平らに開き（Fig. 2. D），これをヘマトキシリンとサフラニンで染色し，脱水した後バルサムで封じた。

　3.　又一方，葉の成長に関する外部形態的な観察は，試料の採集と同日に着葉のまま測定を行った。即ち典型的な成長を示す 20 葉をえらび，5 月 16 日，6 月 1 日，及び 6 月 16 日に Fig. 1 に示すような目盛をつけて，その各部の伸長を測定した。

観　　　察

A. 葉身の成長の観察

　4 月 7 日に初めて鱗片葉が開き始め葉の先端が姿をあらわしてから以後約 2 ヵ月間は，ほぼ一定

の伸長度を示し，6 月 30 日に平均 130 mm に達
して伸長を終った（Fig. 1）。この間 5 月 12 日に
葉長平均 40 mm に達した時期にその長さを 20 等
分して目盛をつけ，その伸長度を観察し続けたと
ころ，先端部から中部にかけては大きな伸長は認
められず，殆ど最基部だけが著しい伸長を示す。
6 月 1 日に到り最基部を 4 等分し，6 月 16 日に
再度その最基部を 4 等分したところ，何れの場合
も等しく最基部だけが著しい伸長を示し，この現

象は葉身の伸長の止る時まで常に続けられるのを
見た。結局最初に 20 等分した最基部は，遂には
全葉長の 1/2 に達する。勿論このような現象は目
盛をつける以前からも同様に行われていたことが
推察される。

　一方，葉幅は葉が長さを増大している間にも極
めて徐々に増大するが，葉長が最大に達した頃か
らやや活潑な増幅がおこり，7 月 21 日に至りその
最大（平均 5 mm）に達したので，この時測定を
止めた。即ちコウヤマキにおいては
葉が伸長を開始して以来，すべての
発育がその最大に達するまで，実に
100 日以上の時日を要するのであ
る。なお葉の横断面を観察すること
により，葉幅の増大が主として次の
原因によることを知った。即ち裏面
表皮の中央部を占める気孔帯は，若
い段階においては内方に向ってたた
み込まれ，葉裏に深い細隙を作って
いるが（Fig. 2. B），成長の段階が
進み葉長が最大に達する頃，気孔帯
の表皮細胞，及びそれに接する葉肉
細胞が切線径を増す方向に成長を行
い，これに伴って気孔帯が開き，横
断面において半円形に入りこんだ気
孔溝を作り，この際に最も著しい増
幅がおこるのである（Fig. 2. C）。
またこの際に海綿組織の細胞間隙も
著しく発達する。

B. 成熟葉における厚膜細胞の分布
　コウヤマキにおいては厚膜細胞は
葉肉部だけに存在し，横断面では主
として柵状組織と海綿状組織の境に
散在する（Fig. 2. B，及び C. ×印
は厚膜細胞の多く存在する位置を示
す）。成熟葉を KOH で処理した後，
Fig. 2. D のように開いたものにつ
いてその全長にわたり観察を行った
ところ，まず一見して次のことがわ
かった。即ち葉の先端部に近いほど
厚膜細胞の数が多く，その樹枝状の
突起部も長く，かつ数が多く複雑な
形状を示し，近接する厚膜細胞と互

Fig. 1.　Elongation curve of the needle-leaf. On
May 12th, it was divided into twenty equal zones in
marking by Indian ink; the lowest zone was
subdivided into four on June 1st and June 16th.
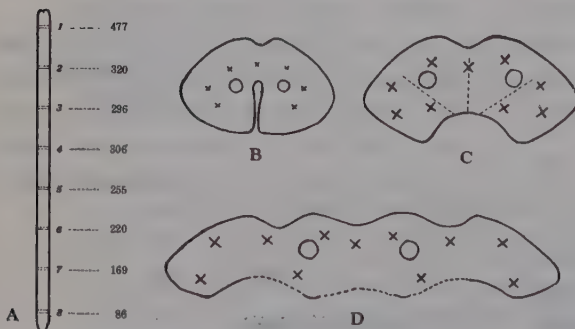
Fig. 2.　A: Showing the number of sclereids
in each zone (1-8; 1 mm in breadth) across the
needle.　B and C: Cross sections of young and
matured needles respectively, × × showing the
distribution of sclereids.　D: Cross section of
matured needle cut open along the broken lines
shown in Fig. C for counting the number of
sclereids shown in Fig. A.

にからみ合っているが（Plate XVII. A），基部に近いほど数が少く，形も単純化され突起部も短い。

更に 10 葉について，Fig. 2. A のような 8 位置で，葉幅全体にわたり幅約 1 mm がよこぎる範囲内にある厚膜細胞の数を数えたところ，同図の右側にあげたような数を示した。勿論正確な数を知ることにはかなりの困難を伴ったが，この値はほぼ同条件の 10 葉についての平均であるから，正しい値に近いものと考えてよいと思う。この結果は上述したことと一致する。

C. 厚膜細胞の発達

厚膜細胞の発生初期から成熟葉に至るまでの発達形態を，各々の縦断切片により日を追って追究すると，4 月 14 日葉長 2.5 mm までの葉では，細胞の形，核の状態，染色の程度等からみても，他の葉肉の細胞と区別することは困難であった。4 月 16 日 4mm になると，厚膜細胞は形の上でやや変化をみせてくる。その最も顕著な 1 例を Plate XVII. B に示す。即ち細胞は他の葉肉の細胞より大きくなり，将来突起として伸びると予想されるやや凸凹のある形を示す。以後葉の発育の進むにつれ凸凹の度が大きくなってくる。4 月 21 日 9.5 mm に達した葉のそれを Plate XVII. C に示す。以後のすべての観察は，基部，中部，先端部の 3 部にわけ，その各部の発達状態を比較した。4 月 28 日 13 mm の葉のそれを Plate XVII. Da（基部），Db（中部），Dc（先端部）に示す。この 3 部を比べると先端部は中部より，中部は基部よりも更に発育の進んだ状態を示す。そしてこれらの厚膜細胞は Plate XVII. Db，及び Dc に示すように，その突起部は近在の葉肉の細胞を包むようにして細胞間隙に伸長する。5 月 6 日 22 mmの葉の厚膜細胞を Plate XVII. Ea（基部），Eb（中部），Ec（先端部）に示す。何れの部位においても Plate XVII. D よりも発育の進んだ状態を示すが，基部（Ea）におけるそれは，前段階の中部（Db），先端部（Dc）のそれよりも寧ろ発育のおくれた状態にあることは，前項の結果からも当然のことである。このような発育の遅速の関係は，以後の段階にも言及できる。5 月 26 日 75 mm の葉の厚膜細胞—Plate XVII. Fa（基部），Fb（中部），Fc（先端部）—は，基部及び中部は未だ発育の過程にあるが，先端部は既に二次膜が肥厚を開始し，近在の厚膜細胞の放

射状に伸びた突起やその切断面が観察される。以後次第に二次膜は肥厚し，本体も突起部も肥厚した膜が大部分を占めるようになる（Plate XVII. A. 成熟葉）。厚膜細胞は葉身に存在する部位により形態的には変化があるが，二次膜の肥厚の程度は同様である。

D. 厚膜細胞の核について

塩酸で解離したプレパラートを用いて，細胞が切断されない本来の形態を追究し，切片のプレパラートと比較検討した。

1. 核の大きさについて．在来の多くの報告は核の大きさを基準にして厚膜細胞と他の葉肉の細胞との判定を行っているが，コウヤマキでは厚膜細胞の核は葉肉部の他の細胞の核と比べて大きさの点からみた差異はない。中にはやや大きいようにみられる例もあるが，それが厚膜細胞の特徴となる程度ではなく，厚膜細胞の発達の進行に当りその細胞自体の著しい発育にもかかわらず他の葉肉の細胞の核の大きさと殆ど差はない。このことは切片のプレパラートによる写真にもよく示されている（Plate XVII. B-F）。

2. 核の移動について．厚膜細胞の枝状部が盛んに伸長している時期のものを多数観察したが，核がその先端に位して枝状部の成長に与っていると考えられる例は全くみられず，ただ厚膜細胞の発達の極めて初期のものではしばしば核が枝状部を形成する僅かの突起に接して存在することが認められた。このことから核の行動について次のことが云えると考えられる。即ちごく発生の初期において厚膜細胞の核は少しずつ移動を開始し，細胞膜に近寄る。核が膜に接すると，ここに枝状部の形成が始まり，緩やかな角度の小突起ができる（Plate XVIII. A，及び C）。更に核は幾度か移動を行い，膜の他の位置と接する度に同様の現象がおこり，細胞は幾つかの角のある形になる。こうして核は幾つかの凸凹を形成すると移動を停止し，本体のほぼ中央におさまる（Plate XVIII. D）。この時期が終ると各小突起は急に伸長を始める（Plate XVIII. E，及び F）。即ちアメーバ型をとり，隣接する細胞の膜間をおし拡げ，細胞間隙に向って突起を伸ばす。なお二次膜の肥厚し始めたものを Plate XVIII. G に示す。つまり若い時期の厚膜細胞の核の移動は，突起形成の初因を誘導し，枝の数

を規定するのに大いに与るが，その伸長に直接に
与ることは殆どないと考えるのが至当であろう。

3. 厚膜細胞の2核について. 厚膜細胞は稀に
2核を有することがしばしば報告されているが，
コウヤマキにおいても極めて僅少な頻度で2核が
出現することが判明した。即ち核の存在する各時
期にわたって，その解離した細胞群の中から厚膜
細胞だけを数え，その中に出現する2核のものを
数えたところ，4月16日（5mm）の葉の解離し
た厚膜細胞 362 中 2 細胞に 2核を認め，4月21
日（9mm）の中から厚膜細胞 523 中2核のもの2
細胞を認めた。

勿論これは単に数を記載しただけで出現率の標
準とならないが，コウヤマキについても極めて僅
少な率ながら出現することが認められた。なおこ
れらの僅少な例については，各々の核の大きさは
何れも比較的不同を示す傾向が認められた（Plate
XVIII. B）。

考　　察

厚膜細胞 sclereids の名称は Tschirch (1889)
が Sklereiden と称したことに始り，以来その種々
な型については幾つかの名称* があるが，Eames
(1947), Esau (1953), Foster (1944-1955) に従う
と diffuse 型の asterosclereids に属する。

葉の伸長に際して，先端部及び中部に比べ基部
が大きく伸長すること，即ち葉の基部の活動によ
って葉の伸長が起ることは，毬果植物一般に見ら
れる現象であるが（Coulter and Chamberlain
1910, Cross 1940 等），コウヤマキにおいてはマ
ツ属の場合と同じくこの現象が特に著しく，最基
部の極端な伸長となって表われる。

厚膜細胞の個々の発達の進行状態は，最近の多
くの研究者（Arzee 1953, Bailey and Nast 1944,
1945, Bloch 1946, Foster 1944-1955, Sacher
1954, Sterling 1947 等）の観察しているところ
とほぼ同様である。ただコウヤマキの研究では，
葉の伸長型と関連して，その葉を先端部，中部及
び基部の3部にわけ，その各々の部位における厚
膜細胞の発達について比較研究した。その結果，
厚膜細胞の成熟は常により先端部がより基部に先

行することが判明した。

Asterosclereid の特性として，突起部の伸長に
際し，隣接する細胞の膜間の中層をおし拡げて細
胞間隙の方に向う。在来成長に際しての隣接の細
胞の相互関係は，sliding and symplastic growth
(Priestrey 1930)，及び intrusive growth (Sin-
nott and Bloch 1939) 等の諸説があり，厚膜細
胞の突起部の伸長と隣接の細胞との関係につい
て，Arzee (1953 b) は Olea において intrusive
growth を主張し，Foster (1944 etc.) は sym-
plastic 及び intrusive growth が相俟って行わ
れることを力説している。コウヤマキにおいては
後者の説を採用するのが至当と思う。

厚膜細胞についての報告の大部分は，その核が
他のそれに比べて大きいことを指摘している。最
も極端な例は，他のそれに比較して 5-8 倍にも達
し，しばしば巨大核ともよばれる。しかしコウヤ
マキでは他の葉肉の細胞の核に比較して殆んど大
きさの差はみられない（Plate XVII. B-F）。やや
大きいものが幾つかあったとしても，核の大きさ
の点から厚膜細胞を判定することは極めて困難で
ある。

又，核が移動を行うことはしばしば問題を提起
し，Arzee (1953 b) によれば Olea において厚
膜細胞の核はその形態形成及び発育に大いに与
り，突起の形成及び伸長に際してその先端部に移
動することを報告している。Foster (1955 b) は
Boronia においてやはり厚膜細胞の発育の進行中
に核が移動することを指摘しているが，決して突
起内部には移動せず，常に本体の内部にあって位
置を変える。この点 Olea と異る。コウヤマキに
おいても厚膜細胞は分化した若い段階にその核が
移動し，幾度か膜辺に寄るが，突起内部に入込む
例はみられなかった（Plate XVIII. A 及び C）。
この移動が突起形成に寄与すると思われるが，突
起が伸長を開始すると殆ど移動しなくなり，ほぼ
中央部に位置する（Plate XVIII. D, E, 及び F）。

Arzee (1953 b), Sterling (1947) 等は夫々厚
膜細胞は稀に2核，及び3核 (Sterling) を有す
ることを報じている。筆者はコウヤマキにおいて
も，極めて僅少な率で2核が出現することを認め

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* Brachysclereids (stone cells), macrosclereids (rod cells), osteosclereids (bone cells), astero-
sclereids (stellate cells), trichosclereids (internal hairs), など。
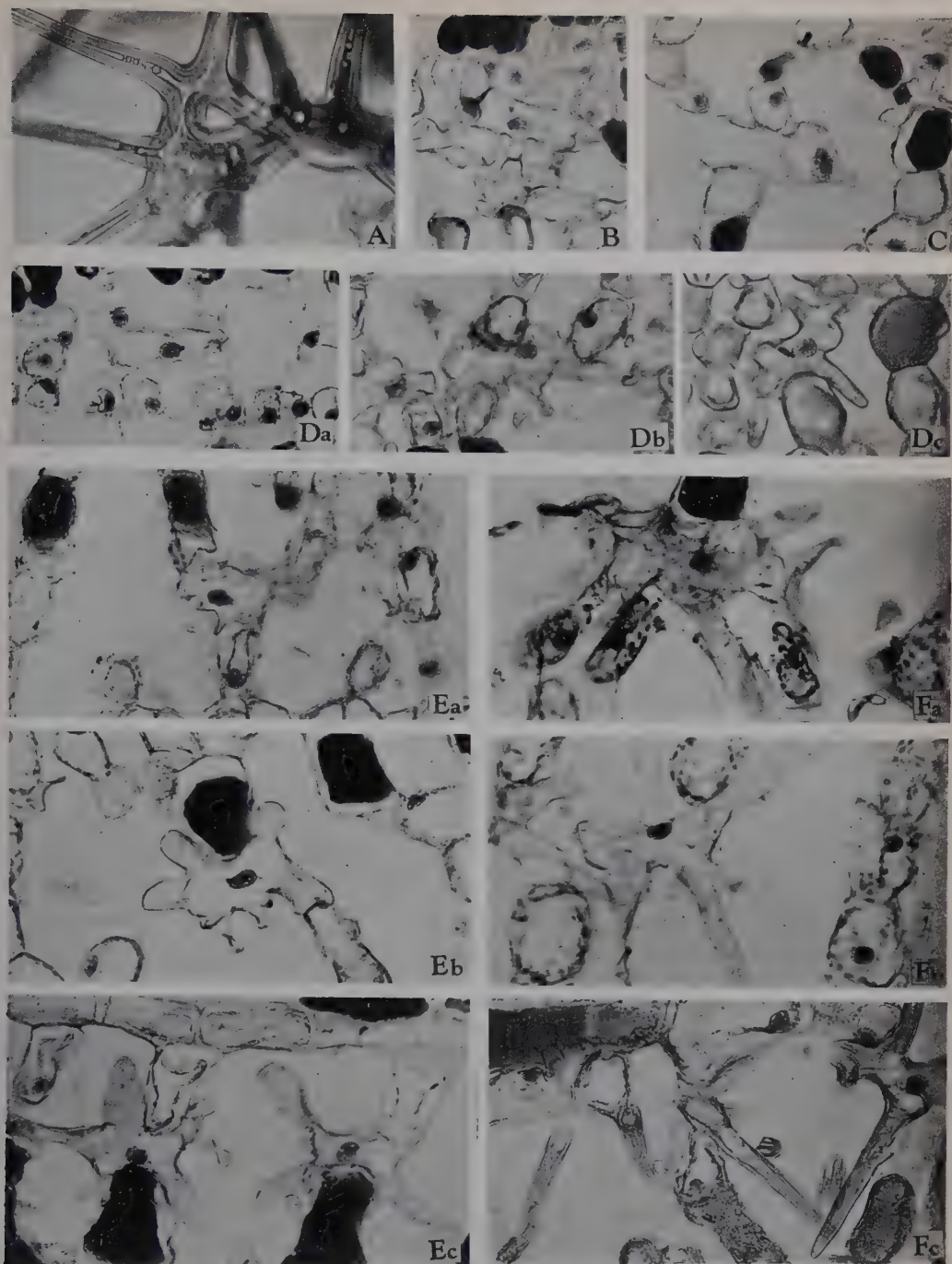
Plate XVII. Various stages of sclereid initials in longitudinal section. Small letters a, b, and c in Figs. D-F indicate basal, middle, and apical parts of the same needle respectively.

A: Matured sclereids. B: Sclereid initial on April 16th (needle 4mm in length).

C: On Apr. 21st (9.5mm). D: On Apr. 28th (13mm). E: On May 6th (22mm).

F: On May 26th (75mm). ×650.

R. Kitamura: Development of the foliar sclereids in *Sciadopitys verticillata*.

Plate XVIII. Development of sclereids. A–F: various types of sclereid initials found in macerated preparation; a binucleate initial is shown in B. G: a matured sclereid. ×1520.

R. Kitamura: Development of the foliar sclereids in *Sciadopitys verticillata*.

た。なおこの場合の両核の大きさは，何れの場合
も Sterling のそれら (1947, Fig. 13) よりも更に
不同であることを示した (Plate XVIII. B)。

本研究に当り，御指導頂いた小倉謙，亘理俊次
両先生に深謝の意を表する。

Résumé

1. Development of foliar sclereids of *Sciadopitys verticillata* Sieb. et Zucc. has been studied in checking for various stages of needle-leaf development (text-Fig. 1).

2. The needle reaches its maximum length after very gradual elongation by the activity of the intercalary meristem during a little over eighty days. After this process, the needle markedly increases its breadth, owing chiefly to the spreading of stomatal groove which is closely folded until that time (text-Fig. 2. B, and C).

3. In matured needle, sclereids are especially numerous and markedly ramified from apex to middle part (Fig. 2. A) while they decrease in their number and become simple in their ramification toward base (Plate XVII. D, E, and F).

4. Differing from many cases hitherto reported, there is no notable differenciation in size between nucleus of sclereid initial and that of mesophyll cell. Accordingly, initial cells are difficult to found until the ramification begins.

5. Translocation of the nucleus of initial cell occurs in certain earlier stages of its development, giving rise the protuberances which soon elongate to form ramifications. There are found, however, no instances in which the nucleus translocates into the elongating branches.

6. Two each examples of binucleate initial were found in at random observation of 326 initials obtained on Apr. 16th, and 523 initials on Apr. 21st (Plate XVIII. B). In both cases, size of the nuclei was unequal.

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クロマツの受精及び前胚の細胞化学的研究

島 村 環*

Tamaki SHIMAMURA*: Cytochemical Studies on the Fertilization
and Proembryo of *Pinus Thunbergii*

裸子植物の卵核及び前胚の核は形が大きいのみ
ならず特殊の構造であり，受精前後の染色質の出
現消長は特異なもので従来注目されている。筆者
は 1935 年にイチョウ，ソテツの受精前後の核の
フォイルゲン反応を研究して発表した。其後核酸
の研究，細胞化学の発達に伴ない再検討の必要を
生じた。材料の関係から先づクロマツについてイ
チョウ，ソテツの場合と比較して核内容の変化を
細胞化学的に調べその結果をここに報告する。こ
の研究の発表に際して，名古屋大学理学部生物学
教室の太田敬久，高尾昭夫両氏の協力によること，
又写真は両氏の撮影によることを記し，こゝに両
氏に感謝の意を表す。

材料及び研究方法

クロマツ *Pinus Thunbergii* は名古屋大学理学
部構内の丘陵に自生せるものの前年受粉した毬果
を 1953-1955 年に亘り 6 月中旬から下旬に採集
した。材料は胚珠だけをとり出して直ちに固定し
た。固定液はカルノア液，ブアン液，テリエスニ
クツキ液，昇汞アルコール混液（50%エタノール
100 c.c. 氷醋酸 10 c.c. 昇汞 8 gr）を用いた。

材料は固定後，水洗，脱水，パラフィン封入し，
ミクロトーム切片にした。切片は 10〜15μ。染色
はフォイルゲン染色，パイロニン・メチールグリ
ン染色及びトルイディンブルー染色を行った。

フォイルゲン反応の加水分解時間は 5〜15 分。
シッフの試薬には 10〜30 時間入れたが共に時間
の長短にによる発色の影響は認められなかった。
パイロニン・メチールグリンは市販のものを柴谷
(1952) の方法により精製調合したものを用いた。

トルイディンブルーは 約 1% 溶液を使用した。
更に酵素リボヌクレアーゼによる消化実験を併用
して染色した。

観　察

中心細胞：　卵核と腹溝細胞核とを 分裂によっ
て生ずる成熟した中心細胞核の核糸（レティキュ
ラム）はフォイルゲン反応正である（第 1 図）。分
裂前期に染色体が核内で著しくよく染色するが，
染色体以外の核物質は反応負である。中期の染色
体はフォイルゲン染色で鮮かに染まる。核内に紡
錘体が見られ分裂像以外の核内容は細胞質に消え
て行く（第 2 図）。

腹溝細胞，卵細胞：　腹溝細胞核と卵核とは最
初形小さく核物質はフォイルゲン反応正である
（第 3 図）。卵核は次第に大さを増して卵細胞の中
心へ向つて降下する。この際フォイルゲン反応は
次第に弱くなる。核が形大となるに伴ない，染色
体以外の物質が増加するためフォイルゲン反応は
負となる。充分に成熟した受精直前の卵核はフォ
イルゲン反応は完全に負である。又トルイディン
ブルー染色では周囲の細胞質及び蛋白質胞（ホー
フマイスター体）は染色するが，核内容は染色し
ない。パイロニン・メチールグリン染色でも卵核
はメチールグリーンで緑色には染らなくて淡赤色
である。これに対し頸細胞核と胚乳（雌性配偶体）
の核は明瞭にフォイルゲン反応正である（第2図）。
腹溝細胞核は細胞質と共に次第に退化して第 4 図
に示す如く痕跡を留める様になる。

受精：　受精は癒合核の時期のみを 観察したの
であるが，雌雄両核共に核内容はフォイルゲン反

* 名古屋大学理学部生物学教室　Biological Institute, Faculty of Science, Nagoya University
Nagoya, Japan.

応負である。たゞ両核の接触した境はしばしば反応正である如く染色した（第6図）。両核が癒合して境が見られなくなった時期のあるものは第5a,5b図に見られる如く　雄核の部分にも雌核の部分にも明瞭に染色された紐状の染色糸が見られる。即ちフォイルゲン反応正である。この期に至って突如として染色性の紐状体が出現するのは驚くべきことである。しかし大部分の残りの核内容は負である。受精後の第一回核分裂の前期，中期（第7図，第8図）を見るに染色体は判然と個体性を示して来る。前期は長くねじれた細い紐状で明かにフォイルゲン反応正である。雌雄両方の染色体が区別出来るゴノメリなる現象は見られない。染色体以外の他の核内容は全部反応負で細胞質と区別することは困難である。但し位相差顕微鏡を用いるとこれを区別することが出来た。中期では分裂像は大きいが癒合核の大さに比すると全く一小部に過ぎない。そこで癒合核内物質の大部分は細胞質に消えて行く。

　前胚：　第一分裂の結果生じた娘核は小さいが次第に大さを増し第二回分裂を行なって（第9図）遊離の四核を生ずる。この四核も生長するに従い核内容はフォイルゲン反応負の物質で充される。この遊離核は四つ共に形大きくなり次第に卵細胞の底部へ降下する。底部に到着して分裂前期に入れば，核内にフォイルゲン反応正なる細い糸状の染色体がフォイルゲン反応負なる核物質で充されて居る核内に散在する様に出現する（第10図）。この状態はイチョウの場合に一局部に塊状となって染色体が出現するのと異なる。この散布した染色体は紡錘体が形成せられるに従って核板に排列する様になる（第11図）。紡錘体は核内に由来するもので核内物質の大部分は細胞質に消えて行く（第12図）。　娘核は最初形小さく次第に大きくなる（第13図）。四遊離核が底部に達する頃，核の周囲にヘマトキシリン，パイロニン等の色素で濃く染色する顆粒が見られる。このものゝ出現はマツの前胚に見られる一特徴である（第12, 14図）。この顆粒の化学的性質は次の如くして決定した。フォイルゲン反応負である（第10, 11, 13図），パイロニン・メチールグリン染色で赤，トルイディンブルーで紫藍色（第12, 14図），ミロン反応で橙赤色，酵素リボヌクレアーゼで消化すると，ミロン反応を除き上記色素で染色しなくなる。以上

の結果からペントース核酸と結合している蛋白質（ヌクレオプロテイン）であると考える。この顆粒は第三回核分裂の際に核の周囲に明瞭に存在して居るが（第12図），前胚が四細胞層になる際には最先端の胚細胞層にのみ著しく見られる（第15図）。フォイルゲン染色は，上記の前胚四細胞層の先端胚細胞層の核は形小さく鮮かに染色して反応正なることを示すが，上部へ向つて各層の核は形大きく次第に染色弱く殆んど反応負と見られる。第16図にては胚細胞の核がフォイルゲン反応正に対して懸垂糸層の核は反応が弱いことが示されて居る。

　以上前胚の各期にてフォイルゲン反応正なることはデスオキシリボ核酸の還元性にて染色することを示し，DNAの所在を示す。パイロニン・メチールグリンの染色ではDNAは緑色，PNAは赤色に染色する。染色体は緑色で紡錘体は赤色であった。

考　　察

　筆者は1935年にソテツ，イチョウの卵核及び雄核に於いてチモ核酸をフォイルゲン反応で検出出来ないことを報告した。今回クロマツの卵核，雄核に於いても同様デスオキシリボ核酸をフォイルゲン染色又はパイロニン・メチルグリーン染色方法では検出されぬ結果を得た。この事実は如何に解釈するか。

　Schnarf (1941) は次の如く見ている。

　核の著しい増大にかゝわらず染色質の量の増加はこれに伴なわない。又染色される物質の構造が若しかすると非常に繊細になってその染色の部分を吾等が見られない程になると云う。筆者の観察の範囲ではフォイルゲン反応負と云うことは顕微鏡下で，ある特定の形態を有するよく染色されるものが見られないことを意味する。デスオキシリボ核酸（DNA）があっても顕微鏡下で観察出来ない程度に分散して居る場合，容積の増大した卵核について定量的にDNAを分析しない限りDNAの存否を如何ともいえない。切片の材料の面積に対して微量に分散されて居るとすればフォイルゲン反応は検出手段としては不適当でありDNAなしと断言出来ない。

　受精卵の癒合核に出現する染色性の紐状体及び核分裂前期，中期の染色体は如何に巨大な核で

あっても必ずフォイルゲン反応正として見られるから，ある一定量の DNA が集まれば顕微鏡下に観察できるのである。

最近同位元素を用いる実験によれば核内物質は絶えず新陳代謝して入替って居ることが証明されて居る。この DNA の糖が一時可逆的化学変化して居りデスオキシリボーズの特性である還元作用が失われる時期があるとも考えられるし，また必ずしも終始一貫して同じ糖の存在を必要としないとも考えられる。しかしこの推論は何等実験的根拠のあるものでなく顕微化学的観察の範囲外である。

次に癒合核から前胚の各期の核内に於ける DNA の量的変化である。この現象は顕微鏡下の観察からも強調し得られるものである。受精後の第一回核分裂の分裂像及び染色体の大さ，長さは続く第二回，第三回の分裂像及び染色体に比して大きく分裂毎に染色体の形，大さ，長さは減小して居る。イチョウの前胚に於てこの現象の見られることを筆者は報告して居る。生物の体細胞核の DNA の量は一定であるといわれて居るが，前胚の核の如く巨大な核が次第にその容積を分裂毎に減じて行く場合 DNA の量のみが変化なく一定であるとは考えられない。事実染色体の大さは分裂毎に小さくなって居り DNA の量も一定でなく次第に減ずることは当然と思われる。

前胚核の大さの減小に関係して，染色体以外の核物質についての変化がある。今仮に分裂像（紡錘体と染色体を含む）に関係する核内物質と其他の残余の部分を形成する核内物質と二つに分けて考えてみると，今前者を idiochromatin と呼び後者を trophochromatin と呼べば核の大さ大なる時には trophochromatin は相当量あり，核が小さくなればその量を著しく減ずる。その割に分裂像は小さくならぬ。換言すれば前胚核の容積の縮少は主として trophochromatin の量の減少で idiochromatin も量を減ずるが比較的減少の度合は前者に比して著しくない。かく二様な chromatin を考える dualism 的な考えは当を得ていないかも知れないが，一つの仮定を提出して見た。四つの遊離核が底部へ降下した頃から核の周囲に多く出現する PNA の豊富な顆粒はペントース核蛋白であると考えられる。このものは将来新しい細胞群が形成される活溌に分裂する核の周囲に見られる。例えば四細胞層形成前，胚細胞形成の最先端の細胞層に特に見られる。最早や分裂せぬ最上層の核の周囲及び薔薇層には見られぬことは注目を惹く。

Summary

1. At an early stage, the newly formed egg nucleus and the ventral canal cell nucleus are Feulgen positive.

2. When the egg nucleus increases in size, it becomes Feulgen negative.

3. In the fused nucleus both the female and male parts are Feulgen negative except the contact part of both nuclei which is Feulgen positive.

4. In some fused nuclei thread-like chromatin filaments show a posifive Feulgen reaction, in both female and male parts.

5. In the prophase of the proembryo long thread-like chromosomes are scattered in the nuclear cavity which is Feulgen negative.

6. The nuclei of proembryo decrease their size after every nuclear division. The chromosomes have the same tendency, therefore the quantity of DNA of each nucleus is not constant and it seems that it gradually decreases according to the growth of the proembryo.

7. Granules, rich in pentose nucleic acid, appear around the nuclei of the proembryo before the four-tier stage and embryo formation.

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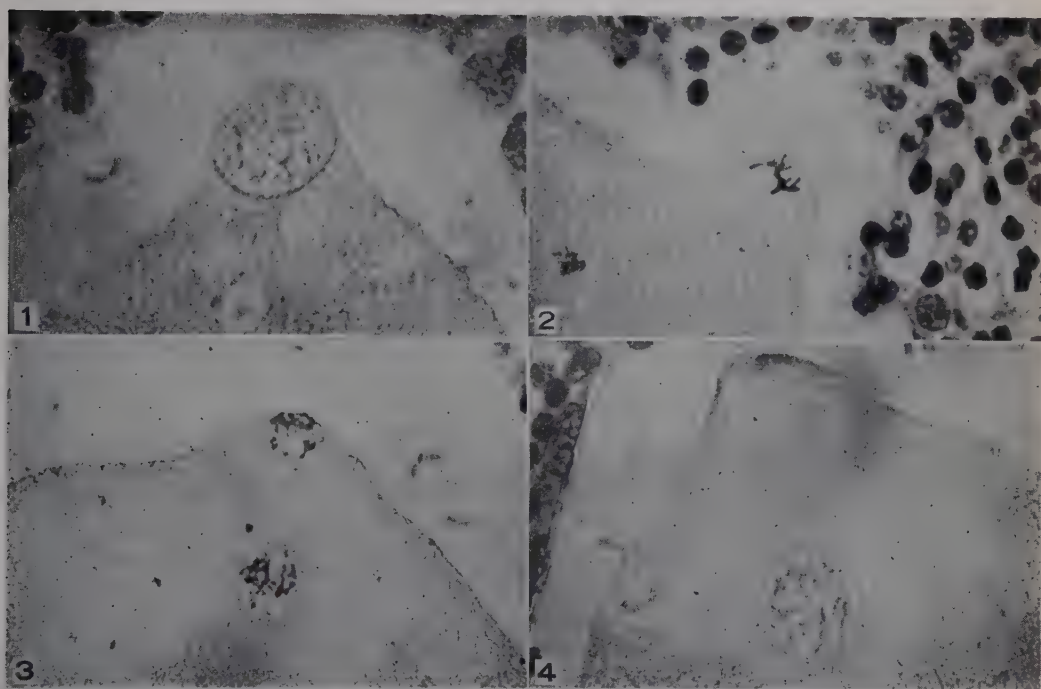
Explanation of figures 1-4. *Pinus Thunbergii.* Cut off of the ventral canal cell, Feulgen nucleal reaction.

Fig. 1. Central cell nucleus before division showing the nuclear reticulum faintly Feulgen positive.

Fig. 2. Metaphase, chromosomes are strongly Feulgen positive while spindle is Feulgen negative.

Fig. 3. Egg nucleus and ventral canal cell nucleus are Feulgen positive.

Fig. 4. Egg nucleus increases in size gradually, moves down and becomes Feulgen negative. In the upper part the degenerate ventral canal cell is shown. × 430.
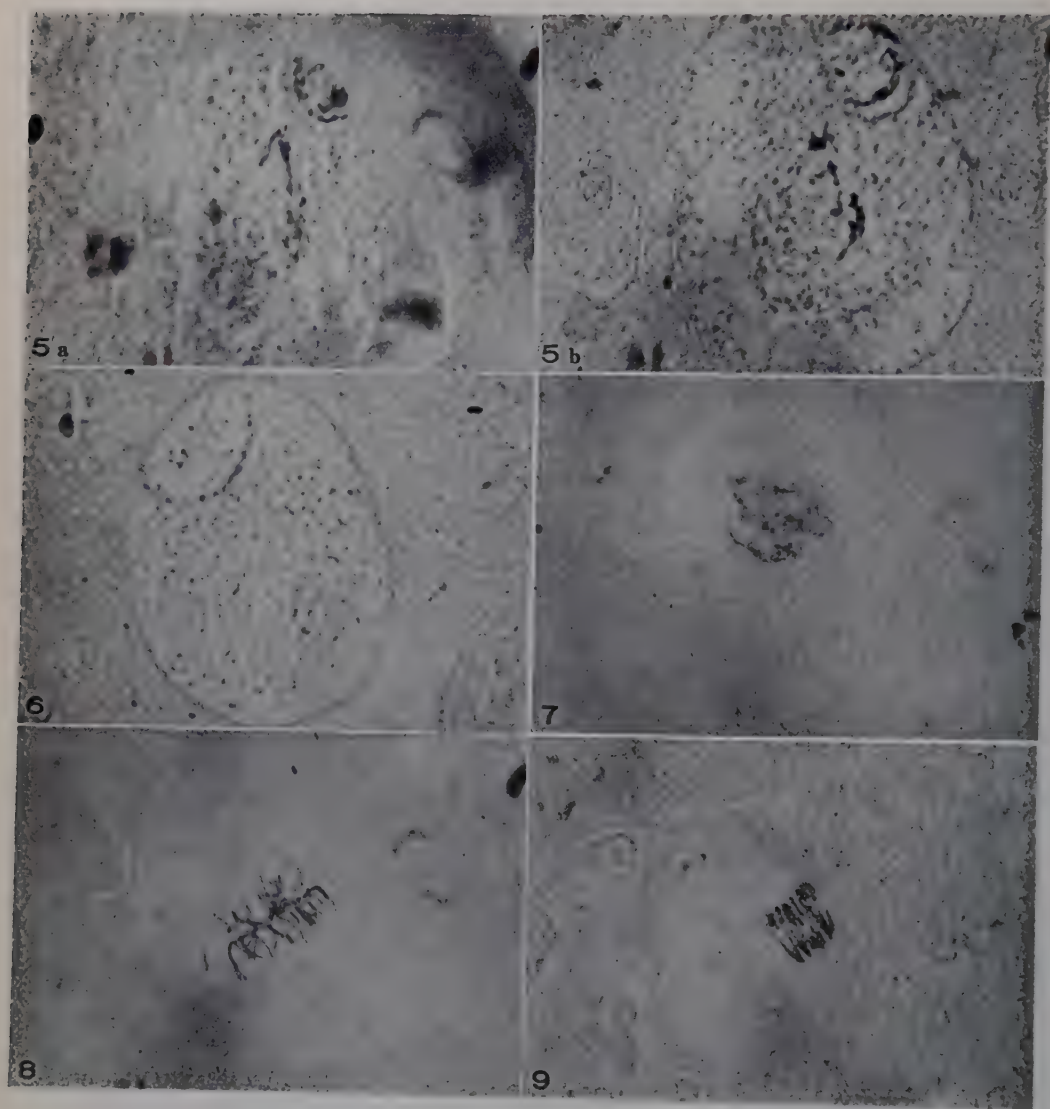
Explanation of figures 5 a–9. *Pinus Thunbergii.* Feulgen nucleal reaction.

Fig. 5 a. 5 b.　Consecutive sections of fused nucleus, the thread-like chromatin filaments show Feulgen positive. The most part of nuclear content is Feulgen negative.

Fig. 6.　Fused nucleus showing both male and female parts are Feulgen negative except the contact part of both nuclei which is stained.

Fig. 7.　Prophase of first division, long slender chromosomes are Feulgen positive.

Fig. 8.　Metaphase of first division, chromosomes arranged on the nuclear plate.

Fig. 9.　Anaphase of second division, mitotic figure is much smaller than that of the first division.　× 430.
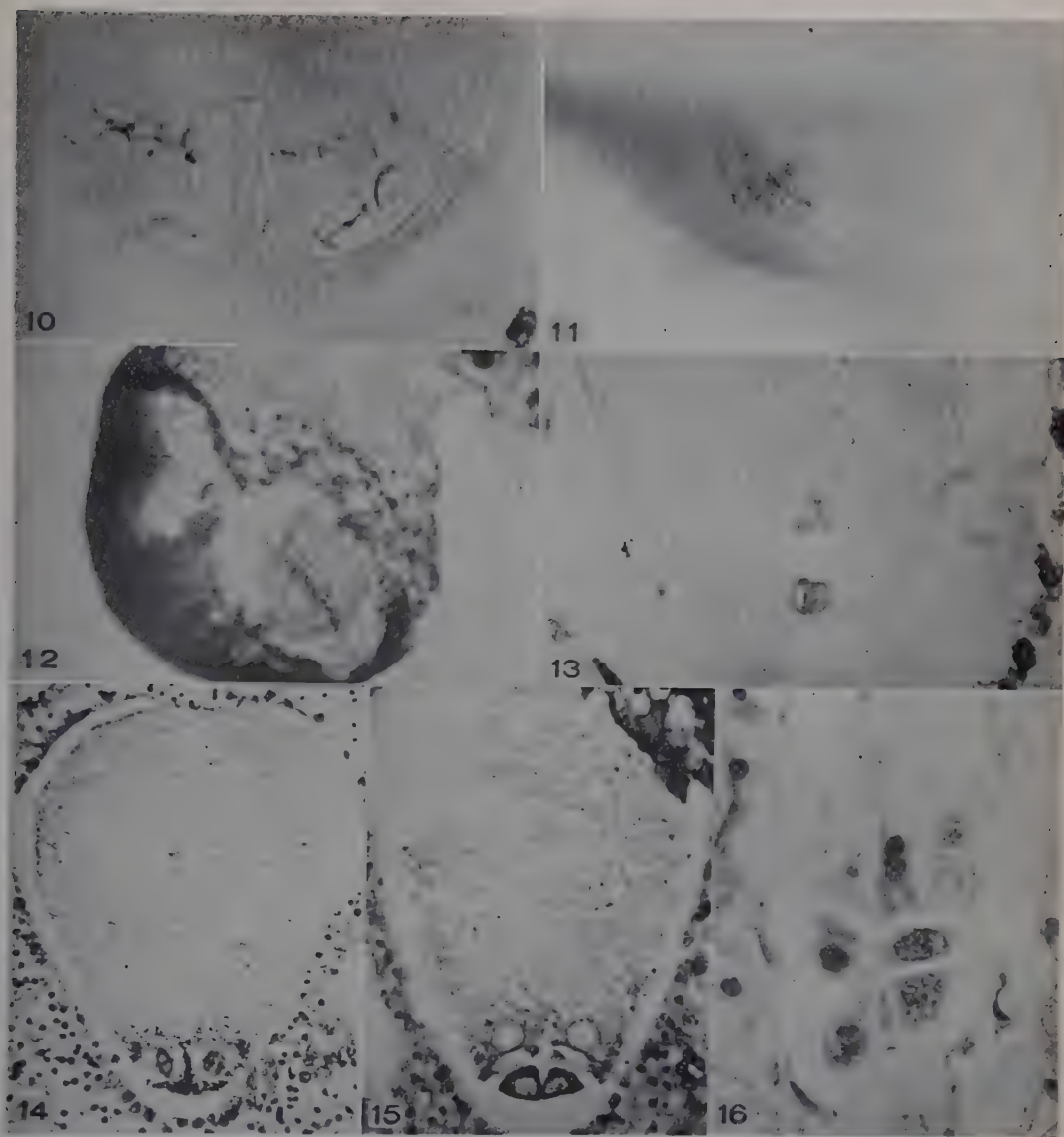
Explanation of figures 10–16. *Pinus Thunbergii.* Proembryo.   Fig. 10. Prophase of four nuclear stage, chromosomes are scattered in the nuclear substance which is Feulgen negative.   Chromosomes are Feulgen positive.   Fig. 11. Early metaphase of mitosis of four nuclei. Scattered chromosomes, going to arrange on the nuclear plate. Fig. 12. Third division of proembryo. Toluidin blue staining. The granules around the nuclei including mitotic figure are well stained. Fig. 13. Telophase of third division. Feulgen nucleal reaction, the granules around mitotic figure are Feulgen negative while the daughter nuclei are Feulgen positive. Figs. 14, 15. Toluidin blue staining shows the distribution of pentose nucleic acid rich granules in different stages of proembryo.   Fig. 16. Suspensor cell and two embryo cells showing the different staining reaction of the nuclei.   Feulgen nucleal reaction.

空中窒素固定能を有する藍藻の米の収穫に及ぼす影響

渡 辺 篤*

Atsushi WATANABE*: On the Effect of the Atmospheric Nitrogen-fixing Blue-green Algae on the Yield of Rice.

I 緒 言

　藍藻のある種のものに空中窒素固定能のあることは可成り古くから唱えられている[1]。最近にはこれらの藍藻を用いて，窒素固定に関する生理化学的研究が盛んに行われるようになった[2]。

　筆者は昭和 16 年以来，南方諸地域例えばインド，ビルマ，タイ，マレイ，ボルネオ，セレベス，スマトラ，ジヤバ，パラオ，フィリッピン等より試料約 600 個を得，その中から 10 数種の空中窒素固定性の藍藻を分離し得た[3]。これらの藍藻について生理化学的研究を行ったが[4]，一方応用的見地からこれら藍藻の水稲の生育に及ぼす影響について実験を行った。この研究の第一報[5]は昭和25 年に発表したが，その内容の主なる点はボルネオ産の *Tolypothrix tenuis*，パラオ産の *Calothrix brevissima* 及びスマトラ産の *Amabaenopsis* sp. の三種の藍藻を稲の水耕及びポット栽培に作用せしめたところ，最高の固定能を有する *Tolypothrix tenuis* の場合にのみ対照の稲よりも草丈，穂数及び穀実の重量に於いて優れていた。この実験は西垣晋及び高田秀夫の両技官によって，農林省農業技術研究所の温室に於いてポット試験で追試された。その結果を推計学的に検討すると有意水準 1 ％に於いて *Tolypothrix tenuis* 及び *Calothrix brevissima* は稲の草丈及び収穫物全窒素に影響があり，特に *Tolypothrix* が *Calothrix* よりも影響

の大きいことが実証された。昭和 27 年に至り農林省振興局の援助によって現地試験が実施された。本報文は 27 年より現在まで 4 年間に各地の農業試験場及び大学の圃場に於いて実施された結果を纏めたものである。筆者がこゝにこの綜説を書くに至った理由は，この現地試験に用いた藍藻は筆者が 10 余年前ボルネオの試料から分離した *Tolypothrix tenuis* であり，且つ 4 年間各地の圃場に配布したこの藍藻は始んど全部筆者の研究室で培養したものであることと，農学専門家による31 年度の結果も入れた完全な報告は可成り遅れる見通しなので，中間報告の意味で簡単に綜説することの必要を認めたにある。現地試験の実施方法は実施者の意向によって多少変化があるが，詳細は各実施者の年次報告を参照して戴くことにして，こゝでは大局的に見て *Tolypothrix tenuis* を圃場に接種することによって，無接種区に比較して粒の増収を来すかどうかという点を明らかにすることに主力を注いだ。

　この研究を援助された農林省振興局に感謝するとともに，貴重な報告の引用を許された各圃場試験実施の各位に深甚な謝意を表する。

II 実 験 方 法

A) 接種用藍藻の多量培養

　実験に用いた藍藻：*Tolypothrix tenuis* は無窒素培養液（K_2HPO_4 0.3 g, $MgSO_4 \cdot 7 H_2O$ 0.2 g, $CaCl_2$ 0.05 g, $FeCl_3$ 痕跡, H_2MoO_4 痕跡, 水 1 l）に接種して，温室（25° C）で日光に当てると空中

* 成城大学生物学教室： Biological Laboratory, Seijô University, Setagayaku, Tokyo, Japan.

窒素をとってよく繁殖し，2 ケ月間に 100 cc の培養液で 5.2 mg の遊離窒素を固定する。もしこの培養液に 0.2％の葡萄糖を添加すると繁殖は更に良好で，同期間中に 9.6 mg の遊離窒素を固定する。この藍藻を圃場に撒布する際の接種量は坪当り 3 乃至 80 g（生体重）であるので，10 ケ所余の試験地に藍藻を補給するためには田植の前後に 10 数瓩の藻体を準備する必要が起る。このため多量培養の方法を工夫した。先ず試験管に培養した藍藻を梅沢式振盪瓶に移植して，照射式振盪培養装置で培養した。10 日間 30℃ で振盪培養すると藍藻は瓶中で粉状に生育する。次にこの培
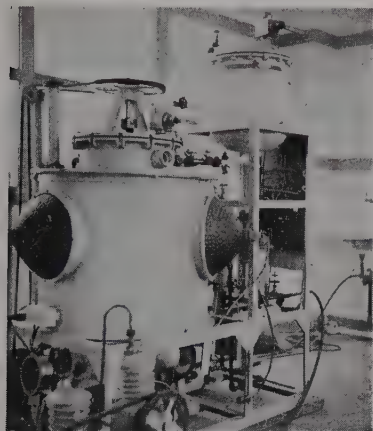
第 1 図　電燈照射式藍藻培養タンク

養液を無菌的に電燈照射式培養タンクに移し，32°C に保温してタンクを運転すると，1 日に約 22 g を増殖する。

次にこのタンク培養液を屋外に装置した大型ビニールチューブ式増培装置に移す。このビニールチューブは長さ 6 m，巾 1 m で中に約 300 l の
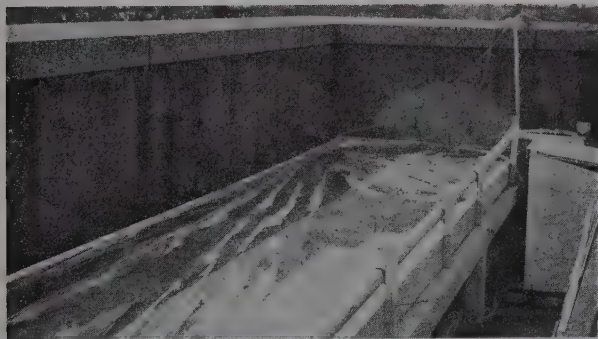
第 2 図　大型ビニールチューブ式藍藻増培装置

培養液が入る。太陽光線照射の下で 3％ CO_2 含有の空気を通じて培養すると 1 日に約 180 g の藻体が得られるので，約 3 ケ月の増培装置の運転によって実験に必要な量の藍藻を賄うことが出来る。夏期に於いては増培装置から出した藍藻は光線と酸素との不足のため速かに変質枯死する場合が少なくなかったので，初年度，次年度に於いては福岡，和歌山等の遠隔地へは航空便を以て培養液のまゝ輸送していた。然しこの方法では輸送費がかさむばかりでなく輸送中に変質が起り易かった。そこで種々研究の結果，藻体を殺菌した鹿沼土と混合して半乾燥状態（含水率 20〜50％）に保つと割合に活力を失うことなく長期保存に耐えることを見出した。現在は主としてこの鹿沼土藍藻を実験材料に供している。

B）　現地試験

現地試験は全国で 11 ケ所（農林省関係の試験場 3，県立農業試験場 5，大学関係の圃場 3）で行った。試験設計は試験地によって又年次によって多少異っている。例えば一区の面積は 1/3300反，1/2000 反，1/60 反，1/40 反，1/15 反，1/10 反等であり，聯数も 1，3，4，6 等であった。又水稲の品種も東海旭，農林 8 号，農林 51号，農林 25 号，農林 18 号等で，土壌も湿田，半湿田，乾田等であった。そこで説明の便宜上一例として鳥取大学の細田克巳教授及び高田秀夫助教授の 29 年度に於ける研究方法及び結果の一部を引用し，次に全試験地の結果を綜合して説明する。

鳥取大学に於ける圃場試験

1）　試験設計

（イ）　使用水田：　鳥取大学農学部圃場（半湿田）

　　（ロ）　1 区面積及び聯数：　1 区面積
　　　　　5.5 坪，4 聯制

　　（ハ）　供試品種：　水稲農林 8 号

　　（ニ）　栽 培 法：　田植え 6 月 25日，1 株 3 本植，株間 8 寸×8 寸，収穫 10 月 27 日

　　（ホ）　試験区の設定：　第一表

　　（ヘ）　施 肥 量：　肥料としての窒素量は慣行施肥量の 2/3 を硫安で施用した。（第二表）

2）　耕種概要

第 1 表 試験区の設定計画

試験区記号	区 名	石灰	窒素追肥	藍藻接種
O 区	標 準 区	—	—	—
N 区	窒 素 追 肥 区	—	追肥	—
Ca 区	石 灰 区	加用	—	—
Ca' 区	石灰, 窒素追肥区	加用	追肥	—
CaA区	石灰, 藍藻接種区	加用	—	接種

第 2 表 試験区の反当施肥量 (貫)

試験区記号	硫 安		過石	硫加	石 灰	
	基肥	追肥	基肥	基肥	第一回	第二回
O 区	5.400	—	9.040	2.960	—	—
N 区	5.400	1.900	9.040	2.960	—	—
Ca 区	5.400	—	9.040	2.960	2.500	2.500
Ca' 区	5.400	1.900	9.040	2.960	2.500	2.500
CaA区	5.400	—	9.040	2.960	2.500	2.500

6 月 19 日: 荒起し

21 日: 施肥

22 日: 代掻

25 日: 田植

7 月 12 日: 1 番除草 (手取り)

13 日: 第 1 回石灰乳撒布 Ca 区, Ca' 区, CaA 区に行う。

14 日: 第 1 次藍藻接種 CaA 区に行う (坪当り 35 g)

8 月 3 日: 第 2 次藍藻接種 CaA 区に行う (坪当り 30 g)

9 日: 第 2 回石灰乳撒布, 硫安追肥を行う。

10 日: 除草 (手取り), 同時に藍藻の鋤込。

9 月 4 日: 穂揃い

10 月 27 日: 刈取り

11 月 26 日: 脱殻調製

3) *Tolypothrix tenuis* の接種及びその水田に於ける生育状況

鹿沼土藍藻を使用した。接種は好天気で気温の高い時に行った。在来の緑藻を死滅させ且つ田面水の pH をアルカリ性とするため接種の前日に消石灰乳濁液を田面水に撒布した。又ミジンコ[8], タニシ等接種藍藻を食害する小動物を殺す目的で, 接種の当日早朝ニッカリン T を 3000 倍になる様に撒布し, 約 4 時間後に藍藻を接種した。接種量は坪当り第 1 次 35 g, 第 2 次 30 g, 合計 75 g であった。接種藍藻は接種当日の午後に於いては可成り気泡を伴って浮き上り, 10 日目頃には相当繁殖して水面に浮び, その後旺んに繁殖して殆んど田面を覆う程度になった。第 2 次接種の藍藻も良く繁殖した。

III 実 験 の 結 果

a) 鳥取大学に於ける試験成績

1) 生育調査: 1 区から 25 株を選定し, これについて生育調査を行った。結果は 1 区当り 100 株平均として示した。

第 3 表 収穫時に於ける生育調査成績
(各区 100 株平均)

区 名	草丈 (cm)	茎数 (本)	穂数 (本)
CaA	102.3	15.3	14.4
Ca	101.9	14.5	13.8
Ca'	101.1	14.3	13.7
N	102.1	15.2	14.3
O	99.5	14.0	12.9

第 3 表にも見られる如く藍藻接種の CaA 区が草丈, 茎数及び穂数ともに無接種の他区に比較して僅かであるが優っていた。

2) 収量調査

全刈り法による各区の全重量及び籾重は下表の如くである。

第 4 表 全刈り法による各区の
全重量及び籾重

区名	風 乾 全 重 量		籾 重	
	区当全収量 kg	収量指数	区当籾重 kg	収量指数
CaA	29.52±0.17	106.23	13.68±0.25	117.93
Ca	27.79±0.56	100.00	11.60±0.14	100.00
Ca'	28.30±0.39	101.84	12.01±0.16	103.53
N	29.46±0.22	106.12	14.01±0.29	120.78
O	26.96±0.17	97.01	11.51±0.17	99.22

表にも見られる如く藍藻接種の効果を CaA 区と Ca 区との比率による所謂増収率で比較考察す

第 5 表　全試験地 4 ケ年間の籾増収率

試　　験　　地[1]	実　施　者	籾　増　収　率(%)			
		27 年	28 年	29 年	30 年
福 岡 県 立 農 業 試 験 場	荻 原 種 雄 技 師	-4[2]	17	56	44 (29)[3]
農林省農業技術研究所 及び 埼 玉 県 立 農 業 試 験 場	西 垣 晋 技 官 渋 谷 政 夫 技 師 坂 井 信 行 技 師 岡 田 親 孝 技 師	2	15	30	
農林省長野食糧事務所 及び信州大学理学部	一 志 未 義 技 官 中 山 包 教 授			22	23
成 城 大 学	渡 辺 篤 石 井 建 次 清 原 千 里		8	21	27
農 林 省 四 国 農 業 試 験 場	平 野 俊 技 官	11	15	20	23
鳥 取 大 学 農 学 部	細 田 克 巳 教 授 高 田 秀 夫 助 教 授	8	10	18	
農林省関東東山農事試験場	城 下 強 技 官 高 橋 和 夫 技 官	0	3	11	(-4)[3]
農 林 省 北 陸 農 業 試 験 場	小 西 千 賀 三 技 官 清 野 馨 技 官	5	2	8	(2)[3]
愛 知 県 立 農 業 試 験 場	藤 堂 誠 技 師	4	6	3	(1)[3]
千 葉 県 立 農 業 試 験 場	佐 藤 吉 之 助 技 師	-1	10	2	10
和 歌 山 県 立 農 業 試 験 場	田 中 秀 太 郎 技 師	-1	-2		4
全 試 験 地 平 均		2.7	8.4	19.1	21.8

1) 試験地の排列順位は 29 年度の増収率の順序に従った。

2) ―は減収率を示す。

3) 藍藻を接種せずして残効調査を行った試験場を示す。(　) 内の数字は残効による籾増収率を示す。

ると風乾重に於いて 6 %, 籾重に於いては 18 ％の増収を示した。然しながらこれらの数字は各個体間の偏差が著るしいので, その確率に問題があるから統計学的に処理をした。2 ケ年の成績を平均して統計学的に処理して分散分析を行った結果, 風乾量では危険率 5%, 信頼度 95% で 6.2% の増収となり, 籾重に於いては危険率 0.2%, 信頼度 99.8% で 12.9% の増収となった。従って水田に対し藍藻の接種は水稲の収量に対し効果のあることが明瞭となった。

b)　全試験地 *四ケ年間の現地試験の成績

* 京都大学農学部の奥田東教授及び滋賀大学の五島喜秋教授によって藍藻の肥効試験が実施されているが, *Nostoc* が使用されたことと *Tolypothrix* の量が少量にすぎたため, 今回の報告からは除いた。尚 31 年度には適量が接種されるので, その成績が期待されている。

試験設計, 施肥量等の異る 11 ケ所の試験地の四ケ年間の成績を端的に纏めるのは可成り困難である。然し詳細の検討は 31 年度の結果も含めて行はれる報告に譲ることにして 27 年, 28 年, 29 年, 30 年度に於いて行われた実験結果のうちから藍藻接種の CaA 区と無接種の対照 Ca 区との比較のみをぬき出して表示した。その結果は上表の如くである。

表にも見られる如く全試験地平均に於いて増収率は 27 年度は 2.7%, 28 年度は 8.4%, 29 年度は 19.1%, 30 年度は 21.8% であった。即ち累年の増収率増加が認められる。この傾向は福岡, 埼玉, 成城, 鳥取, 東山等増収率の比較的高い試験地に於いて一般的に認められる。上表には記載を略したが, 農林省四国農業試験場[9]に於いては昭和 26 年より実験を開始し, 初年度は 4.6

%の増収率を得ているので，5 年間の逐年増加の傾向を最も明らかに認めることが出来る。この傾向は初年度にはその年に繁殖した接種藍藻が全部分解吸収されず，土壌中に潜在窒素地力として残り，次年度には前年度の地力となったものとその年に繁殖した藍藻との累積効果が現われたものと考えられる。

藍藻接種によって水田土壌の乾土効果と窒素潜在地力とが増加することは各地の試験場の報告に明らかであるが，その一例として次に千葉及び福岡県立農業試験場（何れも 29 年度）の報告[10]を抜萃する。

いる。即ち繁殖藍藻体の窒素の約 1/3 量がその年の稲に吸収され残りの 2/3 が翌年の残効となる計算になる。千葉県立農業試験場[12]に於いては 4 ケ年の平均に於いて反当り約 220 匁の窒素が余計に吸収されることを報告している。又鳥取大学農学部の報告[7]によると水田に於ける藍藻接種は反当り硫安 1.9 貫を追肥したものと類似の水稲収量を示している。

埼玉県立農業試験場に於いては 30 年度は藍藻を接種しない接種区跡と対照区との収量を比較する残効試験を行ったが，特に湿田に於いて接種区跡に藍藻がよく繁殖し増收の結果を得た。この実

第 6 表　土壌の乾土効果と窒素潜在地力の増加

区名	千葉				福		岡	
					0～2 cm		2～10 cm	
	水分(%)	乾土効果	NH3—N風乾土中	NH3化成率	水分(%)	アンモニア化成量風乾土100 g 当 mg	水分(%)	アンモニア化成量風乾土100 g 当 mg
CaA	14.78	32.18	41.19	4.63	3.72	20.0	1.70	6.1
Ca	13.01	25.98	36.96	4.32	3.48	15.4	1.64	5.3
Ca'	15.16	24.75	33.33	3.74	3.88	15.1	1.73	5.6
N	12.33	24.88	32.93	4.35	4.09	13.0	1.68	6.1

表にも見られる如く乾土効果及び窒素潜在地力は藍藻接種区（CaA 区）に於いて対照区（Ca 区）よりも価が大きい。

次に表示は略したが，藍藻の接種効果と圃場に於ける接種藍藻の生育状況の間には深い関係があって，接種藍藻のよく繁殖した圃場に於いては必らず肥効が現われ，繁殖しない圃場では殆んどその接種効果が現われなかった。このことは 30 年度に，一部試験地に於いて行った藍藻接種を行わない旧接種区に於ける残効試験に於いて明らかに認めることが出来た。即ち福岡農業試験場[11]に於いては前年度藍藻がよく生育していたので 29%の残効が見られたが，接種藍藻の生育の悪い関東東山，北陸及び愛知の農業試験場では残効が殆んど証明されなかった。

圃場に接種藍藻が繁殖した場合，どの程度の窒素を繁殖藍藻が空中から固定して土壌に与えるかということに関しては，農技研の西垣晋技官の研究[5]がある。それによるとある実験では年間反当り 600 匁の窒素を土壌が得，稲は反当り 225 匁の窒素を余計に吸収していると云う結果を得て

験によって前年度の藍藻が水田に住みつくことが確認された。このことは一度藍藻を接種すると翌年から接種の必要のないことが有り得ることを示している。

IV 考　　察

最も固定能の強い *Tolypothrix tenuis* を接種して行った圃場試験では，接種藍藻が田面水中によく繁殖した場合に限り籾増收の結果が得られている。接種藍藻の繁殖しない場合には全く増收が見られないが，多少とも生育する場合には水田土壌の窒素潜在地力の増強が見られる。

次に接種による籾の増收率は逐年増加する傾向が見られるが，全試試験の平均に於いて 4 ケ年間に最高が約 22%となった。この逐年増加の傾向は繁殖藍藻がその年に全部分解吸収されずして大部分が残効となるためと考えられる。以上は 27 年度より 30 度年までに得られた圃場試験の結果の大要である。

31 年度及びその後の研究の問題点は a) *Tolypothrix tenuis* を接種することにより窒素地

力の増強及び収量増加の起ることを証明し得た
が，今後この藍藻よりも更に強力な固定能を有す
る藍藻が得られゝば肥効効果は今一層高まる見通
しが得られたので，優良品種の探求が望ましいこ
と，b）接種藍藻が繁殖しない時は全く肥効が現
われないので，圃場に確実に接種藍藻の増殖する
方法を確立すること。例えば現在までは田面水の
pH を石灰撒布により中性又は弱アルカリにし，
且つ藍藻食害の小動物駆除の目的でニッカリン又
はホリドールを撒布する方法を実施しているが，
これでは不充分で更に有効な手段を見出すこと，
c）圃場接種に要する藍藻の量は反当り 1 乃至 10
数瓩を要するが，一般農家に配布する必要の起っ
た場合には安価に供給する必要がある。このため
には藍藻の経済的多量生産の研究が必要になる。
藍藻の量産に関係のある事項は藍藻の優良品種の
探求，繁殖率の良い培養液の研究，日光の利用，

安価な CO_2 源の利用，培養槽保温の経済的熱源
の獲得等である。この目的のため目下天然瓦斯利
用及び炭酸温泉利用による藍藻の経済的量産の実
施が考えられている。

V 結　　論

空中窒素固定能を有する藍藻 *Tolypothrix tenuis* を田植え前後に水田に接種すると，接種藍藻
が田面水中に繁殖した場合には籾の収量が無接種
の場合に較べて増加する。その増収率は 11 ケ所
の圃場試験の平均に於いて初年度 2.7%，次年度
8.4%，3 年度 19.1%，4 年度 21.8% と逐年増加の
傾向が見られた。これは初年度にはその年に繁殖
した接種藍藻が分解吸収されず，土壌中に潜在窒
素地力として残り，次年度以後は前年度の地力と
なつたものとその年に繁殖した藍藻との累積効果
が現われたものと考えられる。

Summary

An atmospheric nitrogen-fixing blue-green alga, *Tolypothrix tenuis* from Borneo, was applied on the rice plant in paddy field experiments. In these experiments it was examined whether or not the inoculation of the paddy field with *Tolypothrix tenuis* which is a powerful nitrogen-fixer was conducive to higher yield of rice, through the contribution of the plant remains to the increase in soil fertility. These experiments were done in eight agricultural experiment stations and three universities during the past four years.

As a result of applying this blue-green alga, the yield of rice increased by 2.7 % in the first year, 8.4% in the second year, 19.1% in the third year and 21.8% in the fourth year, on an average of eleven fields. In the first year, only the one-third of the algae which multiplied in the paddy fields was decomposed and absorbed by the rice plants and the remains were contained as the nitrogen fertilizer in the soil. This is the reason why the effect of the algae on the yield of rice increases year by year.

In one experiment, it was made clear that the effect of the inoculation of the paddy field with this alga was almost similar to that of manuring it with 64 pounds of ammonium sulfate/acre as the additional fertilizer.

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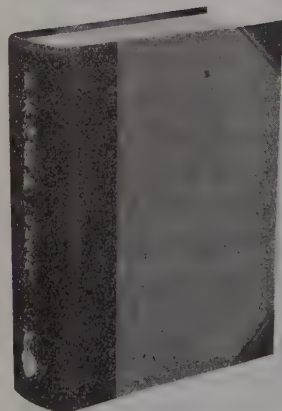
Zur Geschichte der Angiospermen

von Richard KRÄUSEL*

Mit vollem Recht lassen wir das „Mesophytikum", das Zeitalter der Angio-
spermen, mit der mittleren Kreide beginnen, treten uns doch erst von da ab ihre
Reste in grösserer Zahl entgegen. Dies gilt von Di- und Monokotyledonen in gleicher
Weise. Manche dieser Kreideformen lassen sich mit ziemlicher Sicherheit in noch
heute lebende Familien oder Gattungen einordnen. Andere dürften ausgestorben sein,
doch ohne dass an ihrer Angiospermen-Natur Zweifel bestünden. Eine schroffe Kluft
trennt diese ältesten, bereits überraschend formenreichen Angiospermen-Floren selbst
von denen der untersten Kreide, die nach ihrer Zusammensetzung aus Farnen,
Schachtelhalmen und mannigfachen Gymnospermen noch durchaus dem "Mesophy-
tikum" zuzurechnen sind. Aber ist dieser „Florensprung" ein wirkliches Phänomen
oder wird er nur durch eine Lücke in der Fossilüberlieferung vorgetäuscht? Ohne
Zweifel führt der Formenreichtum der kretazischen Angiospermen ebenso wie das
gleichzeitige Erscheinen ihrer beiden Hauptgruppen zu der Vermutung, sie müssten
damals bereits eine längere Entwicklung hinter sich gehabt und schon im Jura oder
gar noch älteren Zeiten vorhanden gewesen sein.

Tatsächlich liegen Angaben über solche mesophytischen Angiospermen vor.
KUHN, der hierzu neuerdings einen Beitrag geliefert hat, bemerkt zwar (1955, b, 164),
dass sie bisher und besonders in den Lehrbüchern noch nicht genügend Beachtung
gefunden hätten. Prüft man aber das darauf bezügliche Schrifttum, so kann man
den kaum beistimmen. AXELROD (1952, 30) zählt eine ganze Anzahl solcher Fossilien
auf; *Suevioxylon* KRÄUSEL, ein Holz aus dem süddeutschen Braunjura, ist für ihn
„definitely dicotyledonous", während THOMSON (1953, 48) meint, das vorliegende
„Material dürfte genügen, um die Anwesenheit der Angiospermen für die ganze
Jurazeit und darüber hinaus mindestens bis in das Rhät hinein sicher zu stellen".
Und auch der Leser des jüngsten deutschen Lehrbuches der Paläobotanik (GOTHAN
& WEYLAND 1954, z. B. 363) wird den gleichen Eindruck gewinnen.

Auch ich neige zu der Ansicht, dass die Angiospermen der mittleren bezw.
unteren Kreide nicht die ältesten gewesen sind. Ein anderer Ding ist aber, ob wir
vorkretazische Reste kennen, die mit Sicherheit als angiosperm angesehen
werden müssen, für die also jede andere Deutung unmöglich ist. Fast immer sind

* Forschungs-Institut Senckenberg, Frankfurt a. M., Deutschland.

uns nur Bruchstücke des Pflanzenkörpers überliefert, wodurch ihr Aussage-Bereich erheblich eingeschränkt wird. Der Begriff der „Bedecktsamigkeit" (Angiospermie) ist der fruchtenden Region entnommen. Gerade Blütenreste haben sich fossil nur ausnahmsweise erhalten, wenn wir zunächst einmal von ihrem Pollen absehen. Früchte oder Samen sind an sich häufiger, ebenso versteinerte Hölzer, die Masse der Fossilien aber besteht aus Blattabdrücken, die bestenfalls Gestalt und Nervenverlauf erkennen lassen. Beide bieten aber kein den Angiospermen ausschliesslich eigentümliches Merkmal. Netzaderigkeit besitzt schon mancher Farne, dann aber vor allem die nacktsamige Gattung *Gnetum*. Der blosse Abdruck eines *Gnetum*-Blattes wäre von dem vieler Angiospermen schlechterdings nicht zu unterscheiden (vergl. MARKGRAF 1926, 431). Nur wenn auch die Epidermis selbst vorläge und anatomisch untersucht werden könnte, wäre eine sichere Trennung durchzuführen. Holzanatomisch sind die Angiospermen durch den Besitz echter Gefässe, u. a. auch im sekundären Holz, ausgezeichnet. Aber solche kommen wiederum auch bei *Gnetum* und seinen Verwandten vor. Es gibt also Gymnospermen mit Gefässen, wie wir umgekehrt auch Angiospermen kennen, denen sie fehlen. Auch da ist also eine absolute Trennung ohne Kenntnis weiterer Merkmale undurchführbar.

Diese Tatsache wird in der Regel unbeachtet gelassen oder mit Stillschweigen übergangen, obwohl gerade sie bei der Suche nach mesophytischen Angiospermen sehr bedeutsam ist. Nur wenn zu der Netzaderigkeit bezw. den Gefässen im Holz weitere „angiospermide" Merkmale treten, wird man die Diagnose „Angiosperme" mit Sicherheit oder zumindest grosser Wahrscheinlichkeit stellen dürfen.

Unter diesem Gesichtspunkt seien nunmehr die von AXELROD und anderen mit den Angiospermen in Verbindung gebrachten mesophytischen Reste betrachtet. Auf einige völlig zweifelhafte Dinge des älteren Schrifttums wird dabei nicht eingegangen.

Palmoidopteris lapparenti BOUREAU (1954, 145, Taf.) ist ein verkieselter Blattstiel aus dem Alb von Tunis, dessen äussere Gefässbündel nach Verf. an die mancher Monokotyledonen erinnern und wie bei diesen bikollateral sein sollen, während die inneren wie bei den Farnen gebaut sind. BOUREAU sieht in dem Fossil eine Art Zwischen- bezw. Vorläuferform gewisser Monokotyledonen, die danach mindestens zum Teil von den Farnen abstammen müssten. Ich halte diese Deutung der äusseren Bündel nicht für gesichert und den gut erhaltenen Rest für den Stiel eines Farnblattes. Auf Ähnlichkeiten zu *Weichselia reticulata* hat BOUREAU selbst bereits hingewiesen.

Propalmophyllum liasinum LIGNIER (1895, 146, Taf. 7 Fig. 20, 21; 1908, 1, Taf. 1 Fig. 1, 2, 6) ist in „Halbrelief" erhalten, nach LIGNIER der oberste Teil des Blattstieles einer Fächerpalme mit noch daran sitzenden Resten der Blattspreite. Auch GOTHAN & WEYLAND (1954, 367) führen es unter den „Palmae" als *Sabal* ähnlich auf, wenn es „auch noch unsicher erscheinen kann." Ich glaube, dass diese Zurückhaltung noch bei weitem nicht genügt. GOTHAN (in POTONIÉ 1921, 14) hat

darin, mit Recht, wie ich meine, die Ausfüllung einer Rieselspur gesehen. Im Sand-
watt der Nordsee kann man ähnliche Bildungen nicht selten beobachten.

Sanmiguelia lewisi Brown (1956, 208, Taf. 32, Taf. 33 Fig. 2; Abb. 29) aus der
Trias von Colorado, USA, soll ebenfalls ein Palmenblatt sein. Aus seinem kurzen,
dicken Stiel entfaltet sich eine nach Art eines jungen Fächerpalmen-Blattes aus-
geprägt längsfaltige Spreite, deren in der Mitte bis über 1 cm breite Teile sich nach
oben wiederum zugespitzt zusammenschliessen. Zahlreiche parallele Nerven sind
vorhanden, die Länge des Blattes dürfte 30 cm überschritten haben. Aber weder
seine Gestalt noch die Art des Überganges in den Stiel ist palmenartig, beide
erinnern vielmehr an gewisse Ginkgophyten. Freilich ist *Sanmiguelia* erheblich
grösser als andere mesophytische Ginkgoaceen-Blätter. Aber diese bieten uns schon
jetzt so verschiedene Gestalten, dass die Möglichkeit, auch *Sanmiguelia* gehöre
hierher, nicht von der Hand zu weisen ist. Die Epidermis, deren Bau das sicher
aufklären könnte, ist nicht erhalten.

Weitere des Erwähnens werte, Monokotyledonen ähnliche Reste liegen nicht
vor. Die von Gross (1955, 55) mit *Potamogeton* verglichenen Blätter gehören nach
Kuhn (1955 a, 497) vermutlich zu Cycadeen.

Furcula granulifera Harris (1932, 4, Taf. 4 Fig. 1–7; Abb. 1, 2) umfasst
länglichschmale Blätter aus dem Rhät-Lias Ostgrönlands. Meist sind sie gegabelt,
besitzen einen geraden Mittelnerv, von diesem ±senkrecht abgehende Seitennerven
und blind in den Maschen endende Nerven letzter Ordnung. An den schlecht
erhaltenen Spaltöffnungen sieht man eigentlich nur die Nachbarzellen mit nach
innen vorspringenden Papillen, wie sie vielen mesophytischen Gymnospermen zu
eigen sind. Axelrod (1952, 30) bringt *Furcula* in die Nähe von *Sterculia*, was völlig
abwegig ist. Eher kämen gewisse Farne in Betracht.

Ungeria solnhofensis Salfeld (1908, 385, Abb. 1) aus dem Jura von Solnhofen
wird von Axelrod mit *Rhus* verglichen, ist aber in Wirklichkeit das völlig unbe-
stimmbare Bruchstuck eines gefiederten Blattes, dessen feinere Nervatur nicht erhal-
ten ist. Nichts daran weist auf Dikotyledonen. Will man überhaupt eine Vermutung
aussprechen, so kämen nur gewisse Farne in Frage. Auf solche weist ja bereits
Salfeld hin. Besonders sei *Bernoullia* Heer aus dem Keuper genannt (Heer 1887, 88,
Taf. 38 Fig. 1–8; Leuthardt 1904, 152, 38, Taf. 19 Fig. 1–4, Taf. 15 Fig. 3, Taf. 20
Fig. 1,2). Schlecht erhaltene Stücke davon sehen durchaus wie *Ungeria* aus.

Phyllites sp. Seward (1904, 125, Taf. 11 Fig. 5, 6) umfasst zwei spitz-ovale
kleine Blattabdrücke aus dem mittleren Jura Englands mit geradem Haupt- und
grundständigen Seitennerven. Der Nervenverlauf ist also ± handförmig. Die feinere
Nervatur ist nicht erhalten. Seward warnt davor, solche Abdrücke als dikotyl zu
deuten, während sie Axelrod mit *Cercidiphyllum* vergleicht. Mich erinnern sie an
manche als *Hausmannia* zu den Dipteraceen gestellte netznervige Blätter. Man
betrachte etwa Taf. 7 bei Richter (1907), besonders *H. forchhammeri* Bartholin
aus dem Jura Bornholms. Seward erwähnt noch einige ältere Angaben über ähnli-

che Blätter, betont dabei aber, sie seien „ at all events valueless as evidence in support of the existence of Liassic Angiosperms ". Das trifft den Nagel auf den Kopf, auch, wenn sie neuerdings als angebliche Angiospermen wieder ausgegraben worden sind; Seward's allgemeine Bemerkungen zu unserem Thema sind also auch heute noch nicht veraltet.[1].

Sassendorffia benkerti Kuhn (1955a, 495, Abb.; 1955 b, 164; 1955c, 56; 1955 d, 802, Abb.), der jüngste Fund eines zu den Dikotyledonen gestellten Abdruckes zeigt ein etwa fünf cm langes, länglich-ovales, oben zugespitztes Blatt mit dickem, geraden Hauptnerven und entfernt stehenden Seitennerven; von der feineren Nervatur ist nichts zu sehen. Die „ Diagnose " der „ neuen Gattung "—eine solche soll ja bekanntlich die Unterschiede gegenüber älteren Gattungen hervorheben—ist denn auch so nichtssagend, dass darunter eine Unzahl ähnlicher jüngerer Blätter fallen würden. Eine Bezeichnungsweise, wie sie Seward gebraucht hat, wäre auch in diesem Falle den Gegebenheiten allein gerecht geworden. Leider ist eine Nachprüfung des Fossils nicht mehr möglich. Kuhn entdeckte es in einer Privatsammlung, wo es zusammen mit zahlreichen Resten einer typischen Juraflora aus einem Steinbruch im Lias α lag; im Gegensatz zu jenen ist es auf der Halde gefunden worden. Mägdefrau, der eine Bearbeitung der Flora plante, musste von der beabsichtigten Gesteins-Vergleichung abstehen, weil das Stück inzwischen verloren gegangen war. So lässt sich seine wahre Natur nicht mehr feststellen; es gilt auch hier das gleiche wie in den schon behandelten Fällen.

Montsechia vidali (Zeiller) Teixeira (1954, 144, Taf. 1–4) ist zuerst von Zeiller aus dem lithographischen Schiefer Spaniens beschrieben und mit dem kretazischen *Pseudoasterophyllites* Feistmantel vereinigt worden. Teixeira hält es nicht für eine Artikulate, und nach seinen Abbildungen (z. B. Taf. 4 Fig. 1, 1 b) ist er damit auch im Recht. Er vermutet darin eine Wasserpflanze, deren Morphologie „ rappelle celle des plantes supérieures, je dirai même de quelques Angiospermes, comme certains espèces de *Myriophyllum*." Ausdrücklich sei aber bemerkt, dass auch er den Beweis der Angiospermen-Natur noch nicht für erbracht ansieht. Tatsächlich könnte man ebensogut an eine K o n i f e r e mit sehr kleinen Nadeln oder gar an eine G i n k g o p h y t e denken.

Montsechites ferreri Teixeira (1954, 146, Taf. 5, 6, Taf. 7, Fig. 4) kommt mit der vorigen zusammen vor. Es besitzt sehr schmale Achsen, deren spreitenlosen Seitenorgane sich wiederholt aufteilen und mit den Blättern des Wasser-*Ranunculus* verglichen werden, während dickere, in ihren Achseln sitzende Gebilde Knospen sein sollen. Ohne Zweifel liegt eine recht eigenartige Pflanze vor, doch will mir scheinen, dass die „ Blätter " vielleicht Verzweigungen anderer Art sind. Ich könnte

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[1] "Species quoted by writers as evidence bearing on questions connected with the phylogeny of flowering plants have in many cases been determined by authors whose lack of botanical knowledge render their records of doubtfull value, if not positively misleading and pernicious. "

mir sehr gut vorstellen, dass eine Alge vorliegt.

Suevioxylon zonatum Kräusel (1928, 250, Abb. 5-8) ist ein Holz aus dem Braunjura Süddeutschlands von sehr schlechter Erhaltung, das aber neben Zuwachszonen Gefässe besitzt. Es war daher berechtigt, es als „Holz vom Angiospermentypus" zu bezeichnen. Nach dem eingangs über *Gnetum* usw. gesagten ist das aber nicht dasselbe wie eine Angiosperme. Als ich es vor fast 30 Jahren beschrieb, habe auch ich mich ohne Zweifel all zu sehr von seinen Gefässen beeindrucken lassen, immerhin jedoch bereits damals auf die Gnetales hingewiesen, deren Holz „nach dem Angiospermentypus" gebaut ist, ohne dass sie Angiospermen sind. Es gilt hier wie auch sonst wohl, dass ursprünglich als möglich hingestellte Vermutungen diesen Charakter bei der Wanderung durch das Schrifttum verlieren und schliesslich in die Lehrbücher gelangen, um von da als nunmehrige „Tatsachen" fröhlich weiterzu wandern. Um es nocheinmal zu sagen: die Angiospermen-Natur von *Suevioxylon* ist nicht sicher bewiesen.

Sahnioxylon Bose et Sah (1954, 1 ;=*Homoxylon* Sahni) und Verwandte (*Phoroxylon* Sze 1954, *Paradoxoxylon* Kräusel 1955, 22, Taf. 5-6) können zusammenfassend betrachtet werden. Es sind gefässlose Hölzer, meist aus dem Jura, deren erstes von Sahni als *Homoxylon rajmahalense* aus Indien 1922 beschrieben worden ist[2]. Gleichen Alters ist *Sahnioxylon andrewsii* Bose et Sah und Jarmolenko's *H. ugamikum* aus Kasakistan, während sein *H. uralense* kretazisch ist (1939, 234). Drei weitere Arten beschreibt Boureau (1954) aus der Trias von Neu-Kaledonien und gleichen Alters sind. *Phoroxylon scalariforme* Sze (1954) sowie *Paradoxopteris leuthardti* Kräusel.

Sahni verglich sein Holz mit den gefässlosen Verwandten der Magnoliaceen, auch Jarmolenko schliesst sich dem an, wenn auch in äusserst vorsichtiger Weise[3], welche Einschränkung freilich ebensowenig wie im Falle *Suevioxylon* beachtet worden ist. Inzwischen hat sich klar gezeigt, dass nur Bennettiteen für einen Vergleich in Frage kommen, wenngleich auch da gewisse Unterschiede, z. B. die starke Entwicklung des Sekundärholzes vorhanden sind.

Weder die Blattreste noch die Hölzer können sonach das Vorhandensein sein echter Angiospermen im Mesophytikum klar erweisen.

Nun hat aber auch die Mikro-paläobotanik Beiträge zu unserer Frage geliefert, die zahlreichsten wohl durch Reissinger. Es genügt, seine Angaben von 1952 näher zu prüfen, wobei zunächst wieder die Pollen ausser acht bleiben.

Gramineae? (1952, 8, Taf. 1 Fig. 28-36). Nach Verf. „mag man über einzelne unter diesen Resten im Zweifel sein, aber das Gesamtbild lässt wohl keine andere Auffassung aufkommen: es werden gramineenartige Reste gewesen sein". Er nennt Spelzen, Lodiculae, Samenknospen, Narben, Staubbeutel und Samen, diese etwa 1/10 mm lang, „also gewiss wie alle diese Reste sehr klein zu nennen im Vergleich mit

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[2] Der Name *Homoxylon* musste aufgegeben werden, da ihn Hartig bereits 1848 auf ein tertiä-res bezw. kretazisches Holz angewandt hat (vergl. Kräusel 1919).

[3] „—showing at the same time a certain similarity as well with Bennettitales. —Until the discovery of leaves and organs in right connection with the stems of the *Homoxylon* type it is impossible to definitely classify this genus among the true Angiosperms".

rezenten Gramineen ". Von keinem dieser fragwürdigen Gebilde verstehe ich, warum sie gerade mit Gramineen zu tun haben könnten. Sie sind undeutbar.

Blütenteile (1952, 8, Taf. 2 Fig. 1-13, Taf. 3 Fig. 48), ebenfalls aus dem Lias α—. Hier sollen wir Antheren-Hälften, Teile von Staubfaden oder Griffeln, einen „Nabel", Leitbündel gekrümmter Funiculae, Samenanlagen sowie ein Blütenblatt vor uns haben. Dieses ist wohl der einzige dieser Reste, der einer Deutung zugänglich ist. Es soll nach dem Crassulaceen-Typus gebaute Spaltöffnungs-Apparate besitzen. Davon kann jedoch keine Rede sein, vielmehr sind es typische Koniferen-Spaltöffnungen. Wir haben ein dreieckiges, schuppenförmiges Blättchen vor uns, wie es z. B. *Pagiophyllum* besitzt. Von den „bemerkenswerten Samen" gehört der 500 μ grosse (Taf. 2 Fig. 14-16) in den Bereich der Hystrichospaeren, andere (Fig. 23, 24) mögen chitinöse Hüllen anderer Einzeller sein.

„Epidermis-Zellen mit schlangenartig gewundenen Begrenzungs-linien" (1952, 11, Taf. 3 Fig. 26-29) werden auf Samen bezogen. Sie tragen zum Teil „Warzen", selten auch Spaltöffnungen. Einige mögen vielleicht (völlig unbestimmbare) Samenhäute darstellen, könnten aber wie die übrigen ebenso gut Blattkutikeln sein. Epidermis-Zellen mit gewellten Wänden und Papillen (den „Warzen") kennen wir von Farnen, Koniferen, Bennettiteen usw. Soweit die abgebildeten Stücke keine Spaltöffnungen zeigen, besagen sie garnichts. Nur auf der „Gramineen-Samenschale" sind solche vorhanden, aber von typischem Bennettiteenbau! Taf. 3 Fig. 49 zeigt eine „Spaltöffnung ganz isoliert mitten innerhalb der Zellumgrenzung." Wenn das in der Mitte gezeichnete Loch wirklich eine Spaltöffnung darstellt, wäre das eine Anordnung, wie sie manchen Farnen eigentümlich ist.

Diese Auslese mag zeigen, was man von den Deutungen REISSINGER's zu halten hat. Er bildet aus dem Lias auch Pollen von „Sumpfpflanzen u. a." ab (Taf. 3 Fig. 1-10), die er mit *Iris*, *Potamogeton*, Palmen, *Hydrocharis* und einer Reihe weiterer Dikotylendonen vergleicht. Soweit es sich da um Monokotyledonen handelt, sei auf die Feststellung eines so sachverständigen Beurteilers wie ERDTMAN verwiesen (1954, 68), dass sich ihr Pollen nicht mit Sicherheit von dem mancher Gymnospermen unterscheiden lässt. Auch die übrigen werden kaum beschrieben und so schlecht abgebildet, dass man sie besser aus dem Spiele lässt. In verstärktem Masse gilt das von REISSINGER's Angiospermen-Resten aus Karbon und Kambrium. Weitere Pollen aus dem Jura sind auf Juglandaceen, Nymphaeaceen und Magnoliaceen bezogen worden, zuerst wohl von SIMPSON (1937, 637, Abb. 2a, d, e). Auch darüber urteilt ERDTMAN sehr vorsichtig, wenn er zu seinem *Monosulcites magnolioides* (1948, 269, Abb. 11) bemerkt, dass „pollen grains of some recent and fossil Gymnosperms exhibit many points of resemblance to this sporomorpha" und weiterhin, dass die rhätoliassischen tricolpaten Pollen „are similar to pollen grains in certain Dicotyledons of our days (cf. Cercidiphyllaceae, Eucommiaceae, Hamamelidaceae).—There are indications of an occurrence of pollen grains with composite apertures in still older layers; but

this needs further confirmation " (1954, 66 u. f.).[4] PFLUG (1953, 60) bezeichnet eine Anzahl paläophytischer Pollen und Sporen als „ angiospermid ", ohne jedoch zu behaupten, dass sie von echten Angiospermen herrühren. Sie bieten ihm die Unterlage für eine morphologische Ableitung des Angiospermen-Pollens. Dagegen lässt sich kaum etwas einwenden, sofern die verglichenen, einander auf den ersten Blick ähnlichen Strukturen wirklich homolog sind. POTONIÉ & ERDTMAN sind jedoch der Ansicht, dass diese Voraussetzung für die von PFLUG konstruierten Reihen nicht gegeben ist (1954, 328).

Wir sind am Ende unserer Betrachtung. Ganz vorsichtig urteilend, darf man sagen, dass auch die Pollenkunde in unserer Frage nicht wesentlich weitergeholfen hat. Es hat nicht an Versuchen gefehlt, das Fehlen der a priori als vorhanden angenommenen mesophytischen Angiospermen zu erklären. REISSINGER denkt sie sich so klein, dass sie in der Regel nicht fossil wurden. Das leuchtet ebenso wenig ein, wie der Gedanke, dafür den angeblichen Mangel mesophytischer Süsswasser-Ablagerungen verantwortlich zu machen (GROSS 1956, 120). Die Annahme schliesslich, sie hätten in fossilisationsfeindlicher Umwelt gelebt (AXELROD, THOMSON), bedeutet letzten Endes auch nur eine Umschreibung der Tatsache, dass wir über sie nichts wissen. Mit DUNBAR (1949, 546) müssen wir noch immer sagen, dass „ Angiosperms are first identified in the Lower Cretaceous ". Ihre mesophytischen Vorläufer hingegen sind noch immer das wesentlichste „ missing link " in der Geschichte der Gefässpflanzen.

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[4] NAUMOVA hat karbonische Pollen auf *Alnus* und *Myriophyllum* bezogen. Ihre Arbeiten waren mir bisher unzugänglich.

Velocity Distribution of the Protoplasmic Streaming in *Nitella* Cells*

by Noburô KAMIYA** and Kiyoko KURODA**

神谷宣郎** ・ 黒田清子**： フラスモの細胞における原形質流動の速度分布

One of the basic questions concerning the mechanism of protoplasmic streaming is whether the motive force of the flow is located within the streaming proto- plasm itself or whether the driving energy is produced in the protoplasmic system not involved in streaming so that the moving protoplasm is driven passively. This is a point on which there is but little experimental evidence and there is a great divergence of opinions.

For an experimental approach to this problem it is necessary to determine how the velocity of streaming is distributed in the different layers of the flow, for with- out knowing and analysing it a sound discussion of the subject is scarcely possible. As early as 1903 Ewart paid attention to this problem and presented a figure of velocity distribution of protoplasm and cell sap in *Chara* cell which shows that the streaming is greatest in the inner layers of the endoplasm and least at the interface between endoplasm and ectoplasm; some degree of streaming is also shown by the cell sap in the layers adjacent to the vacuolar membrane. It was mainly these facts which led Ewart to believe that the energy for conducting streaming is developed in the endoplasm. His figure, however, does not appear to represent the situation of the intracellular motion exactly.

Since Ewart's report, there have been no experiments done along this line except Kamiya's work (1950) on the Myxomycete plasmodium. Our knowledge about the velocity distribution of the protoplasmic streaming in the cell having a cell wall is therefore as yet extremely meager. The purpose of the present paper is to throw light on the problem of the location of the motive force responsible for the streaming by investigating the intracellular velocity distribution of protoplasm exhibiting rota tion—one of the most orderly in pattern of various types of protoplasmic streaming.

In the following experiments we used cells of rhizoid, 'leaf', and internode of *Nitella flexilis*[1] as materials all of which show a vigorous and typical rotational streaming.

I. Velocity Distribution of Protoplasm in a Rhizoid Cell

The rhizoid cell is especially favourable for observing the velocity distribution due to its comparatively thick plasmasol layer (endoplasm) as well as due to the lack of chloroplasts, a fact which greatly facilitates the observation.   The velocity of endoplasm at different layers can be determined by numerous minute granules with which the endoplasm is replete and which serve as index markers.   As there are in the cell two streams occurring in the opposite directions, there exist also two indifferent zones between them where the flow stagnates.   In order to determine the velocity of movement at different strata, we always placed the two indifferent zones in such a position that one was at the top and the other at the bottom of the cell, and observed the flow in the horizontal, optical section through the axis of the cell. By a simple microscopic observation we realize that all the endoplasmic layer moves together with a more or less equal rate along a very thin, almost imperceptible layer of cortical gel(ectoplasm).   For measuring the velocity distribution at one and the same moment exactly, we resorted in this experiment to the cinematographic technique which was described by Kamiya(1950) when he applied it formerly for a similar purpose.

Fig. 1 represents one of the results thus obtained. Cytoplasmic granules found at a moment in an arbitrary transverse section YY′ were carried along with the stream to the positions shown by the open circles, the mean rate of the granules being 21 μ/sec. This figure demonstrates that there is, if any,

Fig. 1.   Velocity distribution of the endoplasmic streaming in a rhizoid cell of *Nitella flexilis*. (Temp. 23°C.)   A part of the rhizoid cell as seen under the microscope is shown on the right. *w* : cell wall, *g* : plasmagel (ectoplasm), *s* : plasmasol (endoplasm), *v* : vacuole.

an only insignificant velocity gradient in the endoplasm itself. Only in the regions very close to the cortical gel layers, where the velocity is represented with broken lines, an extremely large velocity gradient is found. In other words, the plasmasol layer (endoplasm) is actually not streaming, but just slipping as a whole on the inner surface of the cortical layer.   This is a fact which has not been duly expressed or emphasized before, but is extremely important in looking into the nature of proto-
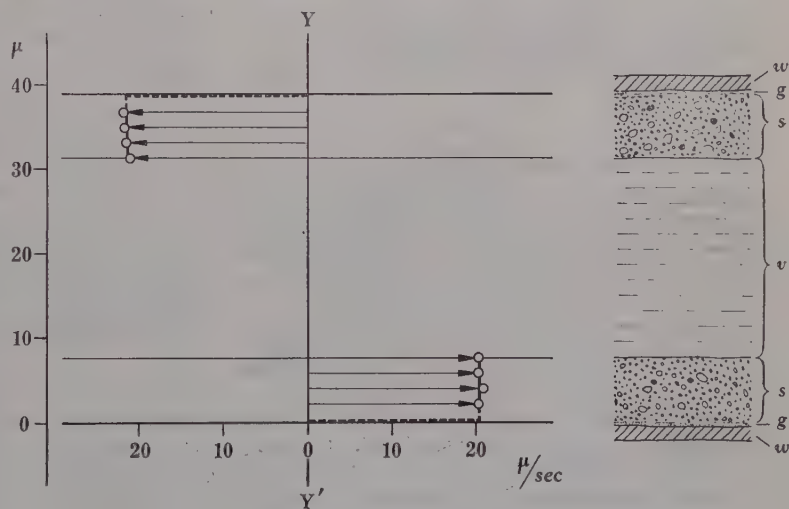
plasmic streaming.

We could not determine the velocity distribution of the cell sap in this case, as there were not enough suspended particles in the vacuole.

II. Velocity Distribution of the Protoplasm and Cell Sap in a Young Leaf Cell

As a young 'leaf' cell is rich in the amount of protoplasm taking part in the streaming and also in suspended inclusions in the cell sap, it permits us to determine the velocity distribution of both protoplasm and cell sap. Furthermore, the situation is simpler than the grown-up internodal cells in that the course of flow is not spirally wound but straight.
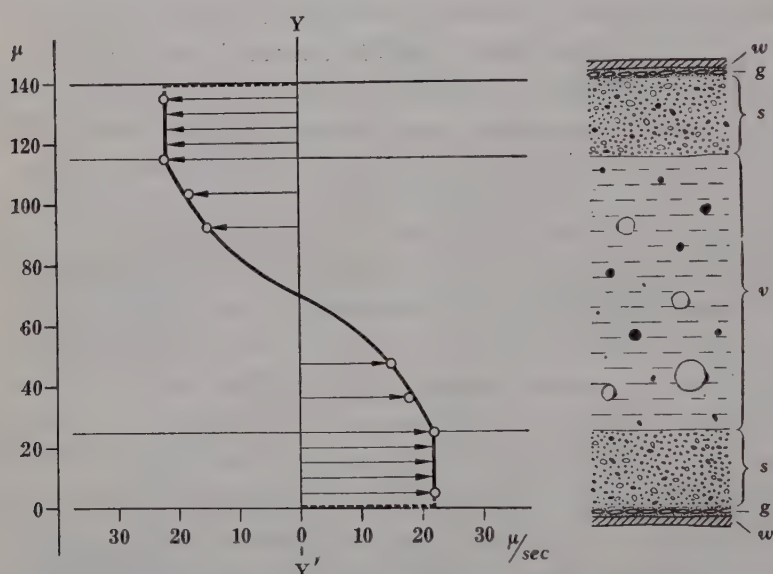
Fig. 2. Velocity distribution of the streaming of protoplasm and cell sap in a young 'leaf' cell. (Temp. 17°C.) A part of the leaf cell as seen under the microscope is shown on the right. w: cell wall, g: plasmagel (ectoplasm), s: plasmasol (endoplasm), v: vacuole.

Fig. 2 shows the intracellular motion in a cell of this kind. The two indifferent zones between the two opposing streams are in this case, too, one at the top, and the other at the bottom of the cell, and the figure represents the velocity in the optical section through the central axis. From this figure we see that the rates of flow at various endoplasmic layers are again the same just as in the case of rhizoid cell in Fig. 1.

The velocity of the sap, which wets the tonoplast and is carried passively by it, decreases inwardly until the central axis is reached where the velocity is nil. On the other hand the velocity gradient of the sap, which is indicated by the slope of the curve against the transverse section of the cell, increases inwardly until the maximum is reached at the point of inflection found at the middle point of the vacuole. The velocity gradients of the cell sap in the peripheral and axial regions of the vacuole were 0.25 μ/sec. and 1.0 μ/sec. respectively per parallel strata 1 μ apart.

III. Velocity Distribution of Protoplasm in a Cell Having no Vacuole

It was reported by Hayashi (1952) that in a strangulated fragment of an internodal cell, the entire space of which is filled with the endoplasm, the protoplasmic streaming still continues, though greatly restricted.

A cell fragment completely filled with protoplasm was made artificially by combining centrifugation and subsequent ligation of the cell (Fig. 3). When an internodal cell (Fig. 3a) is centrifuged gently (1200–1500 r.p.m.) for 10 minutes, the greater part of the endoplasm is forced to the centrifugal end leaving the cortical gel layer behind together with the chloroplasts imbedded in it(Fig. 3b). The amount of plasmasol (endoplasm) taking part in streaming, which may be estimated by this method, was shown in our material to be about 1/5—1/7 of the whole cell volume. If the cell is left untouched, the endoplasm thus dislocated to one end of the cell soon begins to flow back spontaneously along the same course in the same direction as before. In case the centrifugal treatment is strong enough

Fig. 3. The procedure for obtaining a plasm-filled cell fragment, represented diagrammatically. a) One end of an internodal cell of *Nitella* under natural condition. Chloroplasts in the cortical layer are not shown. b) One end of the cell at which the endoplasm is accumulated under the effect of centrifugal force. Arrow indicates the direction of centrifugation. c) A cell fragment produced by strangulation from the centrifuged cell.

as to tear off the cortical gel or to injure its structure, the cell ceases to survive. So far as the cortical gel layer remains intact, however, the forced accumulation of endoplasm at one end of the cell is by no means fatal.

If we tie off such a centrifuged internodal cell promptly with a strip of silk thread near the centrifugal end of the cell before the endoplasm flows back, a cell fragment as long as 10 mm completely filled with plasmasol can be obtained (Fig. 3c). The strangulated cell fragment filled with endoplasm in this manner can grow, if cultured properly, new vacuoles appearing sooner or later (Hayashi, 1952). As a tied-off fragment of the internodal cell keeps all the functions that an intact cell has, we shall hereafter call it simply a cell. Strangulation of *Nitella*-cell has been used recently for elucidating various cell-physiological problems (Sandan, 1955; Kamiya and Kuroda, 1956; Kuroda, 1956).

The cell which is completety filled with endoplasm and has no visible vacuole in
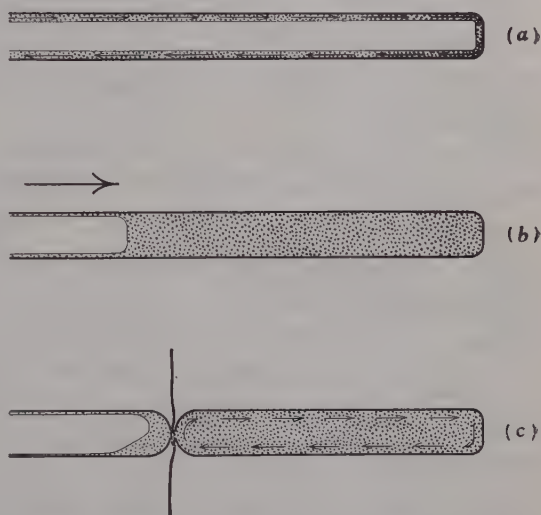
itself showed a rather vigorous streaming in our experiment. This is a fact which shows undoubtedly that the existence of the vacuole is not a *sine qua non* condition for the flowing of the protoplasm. Our purpose now is to determine how the velocity of the protoplasmic streaming is distributed in such a cell.

An example of the velocity distribution is represented in Fig. 4. The cell from which Fig. 4 was drawn was 7.5 mm in length and 0.46 mm in width. The measurement of the rate of flow at different strata was done in this case successively at different time using a stopwatch and an ocular micrometer. However, the flow

Fig. 4. Velocity distribution of the endoplasmic streaming in a cell, the entire space of which is filled artificially with endoplasm. (Temp. 28°C.) A part of the cell as seen under the microscope is shown on the right. w: cell wall, g: plasmagel (ectoplasm) in which chloroplasts are imbedded. s: plasmasol (endoplasm). No vacuole is visible. The rate of protoplasmic streaming in an intact internodal cell was 78μ/sec. at 28°C.

being stable and stationary, we can figure out from the values thus obtained the velocity distribution of the endoplasmic streaming in respect to one and the same moment. The figure represents the velocity of flow in the optical section through the axis of the cell.

It is noticed here that the streams in the two halves of the cell space divided by the central plane connecting the two indifferent striations go in the opposite directions. The two indifferent striations, which take gentle spiral courses (9° against the longitudinal axis) in this case, pass, one on the top, and the other at the bottom of the cell in the transverse section YY′. In the optical section, in which the velocity at different strata was measured, the endoplasm flows either upward or downward one or the other side making an angle of 9° with the optical section. Therefore the actual velocity must be the value of the measured velocity multiplied by 1/cos 9°. This is, however, regarded as being equal to the measured velocity itself, since 1/cos 9° is 1.01. The rate of flow is shown in the figure to be greatest in the peripheral layer and becomes gradually less as the central axis is approached. The inclination of the curve against the transverse plane YY′ is stee-
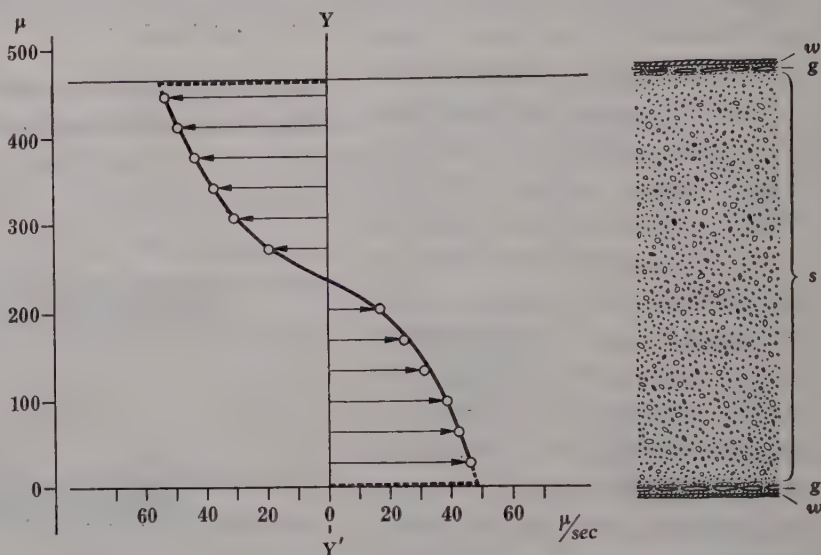
per in the central region of the cell than in the neighbourhood of the peripheries. Thus the curve takes a sigmoid shape with the point of inflexion at the axis of the cell. Here the velocity gradient of the endoplasmic flow in the peripheral region of the cell is 0.1μ/sec. and that in the middle region of the cell is $0.6\ \mu$/sec. per parallel strata 1 μ apart.

In the layers adjacent to the interface between sol and gel, the curve is represented with broken lines as was also the case in the foregoing. This is because the velocity gradient at these regions is so great, that the exact determination of the shape of velocity distribution is impracticable. As shown in the figure, the rate of endoplasmic flow is greater the nearer the periphery is, but it appears that the greatest velocity near the periphery suddenly drops to zero as the cortical gel is reached. The velocity curve is therefore almost discontinuous at both ends near the peripheries.

All in all it is clear enough, as is also the case in Figs. 1 and 2, that the velocity gradient is enormously high in the interfacial zone between the cortical gel layer and the outermost endoplasm. Not only that, but we also become aware that the sigmoid type velocity distribution of the endoplasm shown in Fig. 4 is very similar in form to that of the cell sap in Fig. 2. This is a point which has an important bearing to the mechanism of cyclosis and will be considered more fully in the following.

IV. Model Experiment

In order to demonstrate that the sigmoid type velocity distribution is a necessary consequence of the inner fluid flowing passively when two equal halves of the peripheral zone facing each other in a circular tube are shifted in parallel in the opposite directions, a simple model experiment was attempted instead of trying a tedious mathematical treatment. This kind of model experiment is straight-forward and illuminating in understanding the meaning of the shape of velocity distribution obtained above, and is sufficient for our present purpose.

For conducting the experiment, we prepared a circular, transparent tube made of methyl methacrylate having a bore of 19mm which was cut longitudinally into equal halves having a semi-cylindrical wall. These walls were brought face to face nto a guiding frame so that a complete tube was formed. The construction of the frame is such that it is provided with two projected rails so that the upper and lower semi-cylindrical walls can slide smoothly along them (Fig. 5b).

As a substance to be filled in the tube we found margarine for kitchen use to be best suited for our purpose of observing how far the inner fluid material travels, when the two opposite walls slide against each other. In practice, the right half of the tube was filled with plain margarine and the left half with margarine that was stained red with carmine, and the whole setup was kept at 33°C. so that the entire content became sufficiently fluid; the boundary between plain and red

margarine, however, must be left flat and perpendicular to the axis. The next step was to slide the two semi-cylindrical walls against each other in opposite directions for a definite distance, d, always keeping them tightly on the guiding rails of the frame. Then the set-up filled with margarine was brought into a refrigerator and kept in it until their content got sufficiently hard. We are thus ready to

Fig. 5. Model experiment for showing the distribution of a fluid material in a tube when the two equal halves of the tube were made to shift longitudinally against each other. a) The longitudinal section of the tube after the upper and lower walls were moved from YY' line to Y_d and Y'_d respectively. The space represented by solid circles is filled with margarine stained with carmine and that represented by open circles with plain margarine. b) The cross section of the tube consisting of two sliding semi-cylindrical walls which are placed face to face in the guiding frame on two projecting rails. For further explanations, see text.

remove the walls and cut the cylindrical margarine longitudinally through the median plane ZZ' (Fig. 5b), which is perpendicular to the sliding plane. How this section looks like is shown in Fig. 5a. and Fig. 6.

The similarity of the shape of the boundary thus obtained in this model to the form of velocity distribution of the cell sap (Fig. 2) as well as to that of the protoplasmic flow in a plasm-filled cell (Fig. 4) is striking. The shape of these curves may of course not be exactly the same, because the rheological properties, as expressed by flow-pressure diagram (i.e., velocity gradient—shearing force diagram), of the endoplasm, cell sap, and warm liquid margarine may not be identical. Nevertheless, the fact that the protoplasm in a vacuole-free cell streams with an intracellular velocity distribution which has the same general pat-

Fig. 6. Deformation of the boundary between red and yellow margarine after the upper and lower walls of the tube (removed before the section was made) were shifted against each other. This shows the longitudinal section through the median plane ZZ' shown in Fig. 5 b. The boundary was originally flat and perpendicular to the longitudinal axis.

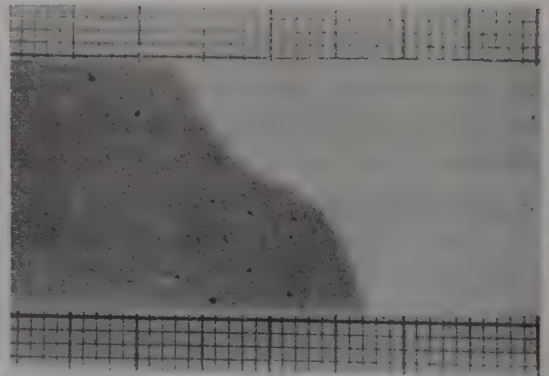tern as that of the deformation curve obtained in the above experiment, or that of the velocity distribution of the cell sap, has a significant implication, namely that the endoplasm moves just like an inert liquid finding itself between two semi-cylindrical walls that are made to slide against each other in the longitudinal direction. This suggests that the endoplasmic flow in Fig. 4. is induced passively. What induces the endoplsm to stream, then, must be the force generated at the interface between plasmasol and plasmagel.

Considerations

It was shown in the foregoing observation that in a rhizoid cell as well as in a 'leaf' cell under natural state, the plamasol (endoplasm) at different layers moves with a nearly equal rate. In other words, there is only a negligible velocity gradient within the moving endoplasmic layer. The movement of endoplasm ,in one direction takes place along the ectoplasmic wall, the cross section of which is semi-circular. The two borders existing between the opposing streams form indifferent zones, which are rather narrow. As the tonoplast is carried with the plasmasol, the direction of its movement across the vacuole is opposite to each other. The cell sap in the vacuole is therefore necessarily subjected to the shearing force between the opposing streams of endoplasm. Hence the situation in the cell sap in the vacuole is comparable, in respect to velocity gradient, to that of the fluid filled in a tube consisting of two sliding walls described above. No wonder that the velocity distribution of the cell sap (Fig. 2) and the shape of the boundary shown in Fig. 6 are very similar to each other.

It is, on the other hand, worth noticing, that the form of velocity distribution of the endoplasm shown in Fig. 4 is also identical:in its basic character to that of the cell sap or of the fluid (warm margarine!) in the sliding wall system. This fact provides a ground for us to conjecture that the endoplasmic motion is also passively caused and the region which plays an active part in driving the endoplasm is located at the boundary between the cortical gel and the endoplasm where the velocity gradient is enormously high.

The fact that the velocity gradient is negligible within the flowing layer of endoplasm in a cell having a large central vacuole is to be understood as being due to the situation that the endoplasm is considerably higher in viscosity than the cell sap. Therefore the absence of the velocity gradient in this case is a further confirmation of the inference that the plasmasol (endoplasm) is inert and it alone can take no active part in the streaming.

Thus all the data obtained by us positively support the view that the active driving mechanism functions only at the boundary zone between plasmasol and plasmasgel. All other portions of the endoplasm, at least in the case of *Nitella* cell, appears to be passive in respect to movement.

The fact that, when the plasmagel layer is injured locally, the endoplasmic

flow no longer occurs on the very spot but goes around it also shows that the special organization in the plasmagel layer is essential for maintaining the protoplasmic streaming (Linsbauer, 1929).

That the plasmasol alone is unable to flow actively is also to be shown by the experiment of isolated naked plasmasol drops from the internodal cell of *Nitella*. By means of a special method of amputation applying a negative hydrostatic pressure to the cell (Kamiya and Kuroda, unpublished), we obtained with least injury very large protoplasmic drops (more than 200 μ in diameter) which can survive more than 50 hours in an artificial medium. What we should like to mention here about these isolated protoplasmic drops which came from the plasmasol part (endoplasm) of the cell, is that they no longer show any rotational streaming as was observed when it was in the cell. This is comprehensible if we consider it as being due to the fact that there is no organized cortical gel layer in these extracellular endoplasmic drops. It is, however, striking enough that a few chloroplasts, which were probably suspended freely in the endoplasm before the cell was cut, show a vigorous rotation each around its own axis. Such a revolving motion of the individual plastids continues usually several hours, sometimes even 2 days. Again, this phenomenon is interpreted neatly as representing the interfacial phenomena between sol and gel. Plasmagel in this case is freely suspended in the endoplasmic drop and consequently what can occur as a result of shearing force acting at the surface of the gel particles (chloroplasts) is not the mass streaming of the sol phase but the individual revolving motion of the gel particles. As a matter of fact a minute, but rapid streaming in the opposite direction is perceptible by a careful observation in the immediate proximity of the surface of the rotating plastids as was also pointed out by Valkanov (1934) in connection with his obserbation of the revolving motion of the nucleus.

The above facts are further in conformity with the conclusion that the interaction of organized gel surface and sol phase produces the shearing force which brings about the interfacial slippage.

The generality of the conclusion obtained by us in *Nitella*-cells is naturally to e checked in other protoplasmic systems. In the case of Myxomycete plasmodium, Kamiya (1950) pointed out that the velocity distribution of protoplasm when it flows in the capillary tube of its own is extremely similar to that of a non-Newtonian liquid transpiring through a rigid circular tube. Furthermore, the endoplasmic flow caused solely artificially in the double-chambar under a pressure difference, gives also a figure of extrusion identical with that of the spontaneous natural flow. This fact undoubtedly indicates that the normal flow o protoplasm in the capillary tube of the plasmodium under natural condition is caused by a local difference in pressure established in the same protoplasmic system. The problem concerning he mechanism of the protoplasmic streaming in the slime mould is, therefore, substituted for the problem as to how a local difference in pressure is established in a proto-

plasmic system.   What comes into focus concerning the rotational movement in *Nitella*, on the other hand, is the mechanism causing the slippage between the sol-gel interface.

The velocity distribution in the form of a flat-headed parabola, as seen in the intracapillary flow of the endoplasm of the Myxomycete plasmodium (Kamiya, 1950), and the velocity distribution appearing in a sigmoid form, as seen in a plasm-filled *Nitella* cell in Fig. 4, are the same in nature in that they are both passive.   Their forms are merely rendered different by the fact that in the former there is a pressure differenece between the anterior and the posterior parts of the tube whereas in the latter the endoplasm finds itself between two semicylindrical surfaces that are made to move in the opposite directions.

The generation of pressure difference occurring locally in a continuous protoplasmic system, as seen in a slime mould, and the generation of a force that brings about a slippage in the interface region between sol and gel layers, as seen in rotational streaming, both belong phenomenologically to the category of "protoplasmic streaming" in as much as they equally bring about streaming.   It remains, however, a question how far they have a common basis in respect to their mechanism.   It is expected that sometime in the future they will be included in one theoretical system.   Though we are really seeking for such a unified picture of movement in protoplasm, it seems at present that there are not enough experimental support warranting such a speculation.   It must be said that we are yet in a stage where we have to investigate each phenomenon separately without any preconceived ideas.

Summary

1) The intracellular velocity distribution of the rotational streaming was determined in cells of rhizoid, 'leaf' and internode of *Nitella flexilis*.

2) In the cells havig a central vacuole, the plasmasol (endoplasm) at different layers flows with a nearly equal rate giving rise to an only insignificant velocity gradient inside.   Only in the very narrow interfacial region between sol and gel layers there is an enormous velocity gradient.

3) In the cell, the whole space of which is filled artificially with endoplasm, the streaming of endoplasm occurs with a velocity distribution which coincides with that we would expect the endoplasm to assume when it is moved passively between two semicylindrical walls which shift in the opposite directions.

4) On the basis of analysis of the intracellular velocity distribution with the aid of a model experiment it was concluded that the flow of endoplasm is passive; the mechanism which drives endoplasm actively resides in the interfacial region between plasmasol (endoplasm) and plasmagel (ectoplasm).

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Anatomical Studies on the Vascular System of
Mirabilis Jalapa L.

by Riukiti Inouye[*]

井上隆吉*:　オシロイバナの維管束の解剖学的研究

The peculiar feature of the vascular system in the stem of some nyctaginaceous plants and of the allied families has been recorded by many writers. It consists in the existence of the medullary bundles and in the thickening growth by the formation of the conjunctive tissue. According to de Bary (1877), in *Mirabilis Jalapa* and *M. longiflora*, only the separate and the fused leaf-trace bundles form the primary and medullary system; other bundles found external to it are produced by the extrafascicular cambium with their surrounding parenchyma cells, though they are apparently medullary, being imbedded in the tissue similar to the pith. Wilson (1924) has given a special remark on this point that in many species of the Amaranthaceae and Chenopodiaceae, the first-formed elements of the conjunctive tissue look like primary cells, so giving an appearance of the medullary bundles to those which are peripheral in reality. Contrary to de Bary, Maheshwari (1930), working on the ontogeny of the stem in *Boerhaavia diffusa*, has reported the presence of the primary bundles in the stelar periphery, and he has also insisted that the conjunctive tissue is not produced by the extrafascicular, but by the normal interfascicular cambium. The formation of the conjunctive tissue by the activity of the extrafascicular cambium which arises in the peircycle is, however, accepted by many

* Department of Biology, Saitama University, Urawa, Japan.　埼玉大学文理学部生物学教室

investigators (de Bary, 1877; Fron, 1899; Solereder, 1908; Artchwager, 1920; Wilson, 1924; Eames and Mac-Daniels, 1946). The present writer (1956a) has pointed out the similarity of the longitudinal course of the main leaf-trace bundles of *Achyranthes japonica* with that of *Mirabilis* described by de Bary (1877), and he (1956b) has also suggested that in *Mirabilis* all the bundles in the stelar parenchyma are probably of the primary nature, basing on his studies on some species of *Amaranthus* and *Celosia*. The present work concerns the course and the development of the primary vascular bundles of *M. Jalapa* for the purpose to interpret the organization of the primary vascular system. The origin of the conjunctive tissue is not considered at present.

Methods All the materials were fixed in formalin-acetic-alcohol; imbedded in paraffin by the usual method; cut transversely at 8–12 μ; stained in safranin, Heidenhain's haematoxylin and fast green. For the ontogenetical study various corresponding levels of several apical internodes in various developmental stages were compared. The more aged nodal regions bearing the axillary branches with fully developed primary tissues were studied to trace the vascular course. To avoid the intricacy caused by the numerous peripheral bundles in such materials, and to follow the course of the inner bundles exactly, the younger nodes were examined.

The arrangement and the course of bundles In a transverse section of an internode in which the primary development is just completed, the central cylinder is clearly defined, being bordered by the pericycle (Figs. 1, 5). It contrasts finely with the large-celled cortex by its narrow and slender cells, but it merges inward into the pith parenchyma, so that the exact thickeness of the tissue can not be determined (Fig. 5 *per*). The leaves are opposite. According to the phyllotaxis, the regular arrangement of bundles is turned at right angles in each successive internode. The bundles are arranged in three concentric rings. The inner ring consists of eight bundles, namely a pair of opposite trifascicular leaf-traces (Fig. 1 *L–M–L*) and two larger ones (*C*) alternate with the former. The latter is the cauline bundles as will be explained later. The cauline bundles bifurcate at the base of the internode; each of the bifurcations fuses the adjoining lateral leaf-trace bundle. The bundles of the leaf-trace descend an internode side by side without any branching or fusion till they reach the node below. Here the median bundle unites both lateral ones which have just united the bifurcation of the cauline bundle. The fused bundle then passes down into the next internode, where it is the cauline bundle.

The second and the third vascular rings are easily discernible in the near of the nodal region, but in the middle of the internode they lie close to each other and the bundles are arranged irregularly to a certain degree (Fig. 1). The bundles in the inner two rings of the axillary branch become arranged in a single ring just before they insert. Upon entering the axis, the ring is dissected into two opposite groups. Each of them passes by the fused bundle just formed at the concerning
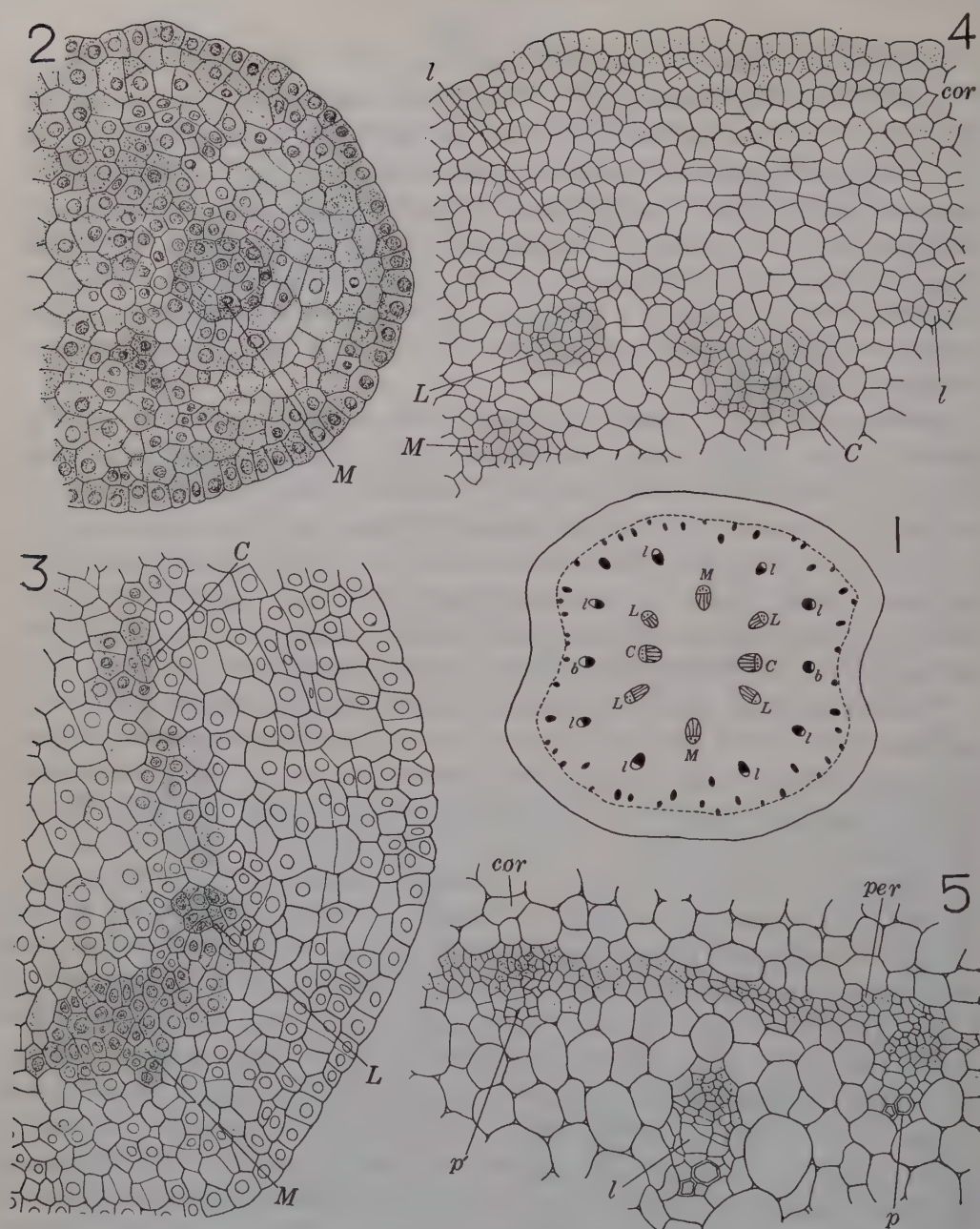
Fig. 1. The arrangement of the primary vascular bundles in the stem, a diagrammatic representation; broken line indicates the margin of the stele.　*L, M, l*, lateral and median main, and intermediary leaf-trace bundles: *C*, cauline bundles; *b*, branch trace bundles; bundles drawn in solid black are the peripheral ones.　×15. Figs. 2-4. Transverse sections through three successive apical internodes, showing stages of the differentiation. Fig. 2. Internode below the highest node. The pith is vacuolated (left).　×350. Fig. 3. Internode next to that shown in Fig. 2, showing the tangential divisions in the tissue external to the cauline bundle (*C*).　×350. Fig. 4. The next lower internode, showing the stage of the differentiation of the cortex. ×220.　Fig. 5. Periphery of the central cylinder with well-defined percycle.　×200.　Notations of bundles in these four figures are as in fig. 1. *cor*, cortex: *per*, pericycle.

node, and then coalescens with the corresponding one from the opposite branch be-
hind each cauline bundle, giving rise to the branch-trace bundle (Fig. 1 *b*). Then
leaf-trace insertion follows. The leaf-trace has four small intermediary bundles
besides three main ones. At the insertion the intermediary bundles are left behind,
and arranged in an arc outside of the main leaf-trace bundles (Fig. 1 *l*), forming
the second ring between the inner and the peripheral ones with the branch-trace
bundles.

The bundles in the second ring descend an internode, then they make a intricated
vascular complex at the lower node, variously splitting and fusing with one
another and also with the bundles of the outermost vascular ring. It was hardly
possible to trace individual courses with certainty. Notwithstanding, it has been
found that the bundles in the outermost ring of the next lower internode arise from
this vascular complex. The bundles of the outermost ring of the lateral branches
join the vascular ring of the same kind in the main axis.

All the bundles of the second ring (Fig. 5 *l*) are separated from the pericycle
by at least one or two cells, which are more alike to the typical medullary cells in
their shape than to those of the pericycle. Hence the second ring is to be consi-
dered as a part of the medullary system. Consequnently it is very natural to con-
clude that the bundles in the second ring are of the primary development. As for
the bundles of the third ring, some are in direct contact with the pericycle, others
are separated by a few cells from it. The intervening cells are intermediate bet-
ween the typical pericyclic and the medullary cells. Such a condition has been
found in the peripheral ring in *Amaranthus* (Inouye, 1956b). It must be remarked
that in these bundles the annular or spiral vessels are confirmed. This shows that
they are of the primary nature as medullary bundles are.

Ontogeny of the bundles In the shoot apex, nearly at the same level of the
differentiation of the pith, there appear two procambia as small groups of highly
protoplasmic cells. They are the median main leaf-trace bundles of the differentia-
ting primodia at the top of the internode. As the differentiation proceeds, the pith
grows wider by the enlargement and variously directed divisions of its cells. The
cells exterior to the procambia (Fig. 2 *M*) begin to vacuolate and increase their
number in radial direction by their tangential divisions leaving a zone of protoplas-
mic cells between the pith and the external tissue. The lateral main leaf-trace
bundles (Fig. 3 *L*) differentiate a little later in this protoplasmic zone. Before the
further differentiation begins, the lower node develops.

In the internode next below all of the eight bundles of the inner medullary ring
are developed. Among them, however, the cauline bundles appear later than
others, and often very poorly defined in younger materials (Fig. 3 *C*). In the tissue
outside the cauline bundles cells are arranged somewhat radially, and many thin
tangential walls are noticed, which indicates that they undergo prevailing periclinal
divisions, resulting in the increment of the radial number of cells up to about ten

or more. Any differentiation of tissues is not remarked within this region at first. Later the cells in the outer layers of this region become markedly smaller than those of the inner region probably by the radial divisions. This region is the cortex (Fig. 4 *cor*). In contrast with this, the tangential divisions continue in the inner layers, so as to produce the the outer part of the pith external to the cauline bundles. At this time an ambiguous tangential row of cells appears just outside the leaf-trace. This is the limitting layer of the central cylinder from the cortex, and the tangential divisions of its cells give rise to the outer part of the pith behind the leaf-trace.

While the primary thickening growth of the pith goes on, the inner region gradually loses its radial arrangement of cells, assuming the feature of the pith on one hand (Figs. 4, 5), and on the other, in the outer region cell divisions continue, when the procambia of the second medullary ring are layed down in this region (Fig. 4 *l*). They become separated from the border of the stele by further thickening growth of the latter, while the procambia of the third ring are produced in the same way as those of the second ring (Fig. 5 *p*). When the primary thickening of the stele is settled, the cells of its periphery are more or less distinct from those of the cortex and of the pith by the small diameter and slender shape (Fig. 5 *per*). The zone of such cells is the pericycle. At places narrow cells are clustered in groups of considerable size in the pericyclic zone. They develop into the bundles which are in contact with the pericycle (Fig. 5 *p'*).

The extrafascicular cambium arises in the outermost cell layer of the pericycle. It is connected laterally with the intrafascicular cambium of such bundles in the peripheral ring that lie close to it. Where the extrafascicular cambium passes outside such bundles, it begins to function after that the intrafascicular cambium has produced a little amount of the secondary tissue, and its activity has ceased. So that such bundles become half imbedded in the conjunctive tissue, which has probably caused the misinterpetation that they are of the secondary nature.

Discussion: The writer (1956a) has brought forward a suppositional original plan, from which the vascular system of the amaranthaceous plants with opposite leaves might have been derived, such as the species of *Achyranthes*, *Iresine* and *Gomphrena*, and he (1956b) has shown that such plants with alternate leaves as *Amaranthus* and *Celosia* have the vascular system which can be explained as a derivative of the original plan. From the viewpoint of the vascular course, a remarkable resemblance is noticed in the organization of the vascular system of *M. Jalapa* and of species of the Amaranthaceae. The behaviour of the cualine bundles in *M. Jalapa* is all the same with that of the medullary bundles of *Achyranthes japonica*, provided that they are arranged in the same ring with the main leaf-trace bundles in the former. It must be remarked, however, that they are situated a little inner to the main leaf-trace bundle, which can be interpreted as an expression of the tendency of such bundles to be arranged in an distinctive inner ring. On the other

hand, in species of *Amaranthus* and *Celosia* cauline bundles form the inner medullary ring with the main leaf-trace bundles as in *M. Jalapa*. Considering the facts mentioned above, it is concluded that the organization of the vascular system of *M. Jalapa* is derived from the original plan proposed for the explanation of the vascular system of the Amaranthaceae.

As for the ontogeny of the bundles, the procambia of all the bundles in concern appear in the periphery of the growing central cylinder before the pericycle is differentiated; in other words, simultaneously with the primary thickening growth of the pith. The same has been found also in some species of *Amaranthus* and *Celosia* (Inouye, 1956b), and in *Boerhavia diffusa* (Maheshwari, 1930). Both of these writers have considered them as the primary bundles contrary to the widely accepted view that they differentiate in the inner part of the conjunctive tissue. The fact that even the bundles of the peripheral ring have spiral or annular vessel elements will support this interpretation.

Summary

In *M. Jalapa* all the bundles situated internal to the conjunctive tissue are of the primary nature. They are arranged in two medullary and a peripheral vascular rings. The inner medullary ring is composed of the main leaf-trace and cauline bundles; the next one, of the intermediary leaf-trace bundles. The peripheral ring consists of the lower continuations of the bundles of the outer medullary ring. Such an organization of the vascular system as this is all the same with that of *Amaranthus*, and can be derived from the suppositional original plan which have been brought forward by the writer (1956a) to explain the vascular system of the amaranthaceous plants. The course of individual bundles is all the same with that of *Achyranthes japonica* as a whole.

The vascular course and the ontogeny show that even the peripheral bundles are primary, as it has been shown by Maheshwari (1930) in *Boerhaavia diffusa* and by the writer (1956b) in *Amaranthus* and in *Celosia*.

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Origin of Copper Resistant Cells

Studies on the Adaptation off Yeast to Copper XV*

by Joji Ashida** and Mutsuo Imai**

芦田譲治**　今井六雄**：　耐性細胞の起源（コウボの銅適応の研究 XV）

The development of resistance to toxic agents in microbes makes an interesting and important problem from various points of view.[1] In this concern, yeast makes an object of special interest, since the method of genetic analysis is available in this organism. The large size of yeast cells makes microscopic observations easy. Although the cluster formation of cells furnishes certain inconveniences in some experiments, a colony grown from a parent-daughter cluster is genetically equivalent to that grown from a single cell.

The results of experiments carried out to disclose the origin of copper resistant cells are reported in this paper.

Material and Method

Saccharomyces ellipsoideus strain K was used as the parent strain. The sub-strain obtained by training the parent strain with the standard medium to which $CuSO_4$ was added (final concentration: 1 mM) was denoted as R_{Ib}. The R_{Ib} sub-cultured in the medium without copper was designated as $R_{Ib(O)}$. When cells of either R_{Ib} or $R_{Ib(O)}$ were plated on the standard agar medium containing 1 to 2 mM of copper, they grew into round and smooth brown colonies. Let this type of colony be called the R_{Ib}–type. On the same copper plate, cells of the parent strain grew into colonies with rugged outlines and surfaces.

The composition of the standard medium, called MH, was KH_2PO_4 5g, $MgSO_4 \cdot 7$ aq. 2g, peptone 5g, cane sugar 100g, water 1 l, wort(Bé. 8) 360 ml. For the copper medium, a measured volume of sterilized $CuSO_4$ solution was mixed with a measured volume of MH at room temperature, or, in the case of the agar medium, at 45° C before solidification.

Results

Observation by plating

Cells were suspended in MH agar at 45° C and a measured volume of this cell suspension was mixed with a small volume of sterilized $CuSO_4$ solution, and aliquots of the mixture were poured into Petri dishes. Thus a series of plates of graded

copper concentrations, including those without copper, were prepared from a cell suspension. For plating the parent strain and $R_{Ib(O)}$, 48-hour cultures were used. The culture of about 60 hours of age was used for R_{Ib}, since the growth was a little slow in the copper medium.

The survival ratio, namely the ratio of the number of visible colonies appearing in the copper plate, to that in the control plate, was calculated. The survival value of a given type of cells in a given copper-concentration varied rather largely from one experiment to another. Figure 1 was prepared by using the averages of such values, in order to obtain a general picture of copper resistance of the three types of cells.

The comparison of curves for $R_{Ib(O)}$ and R_{Ib} in the figure shows that the copper-trained substrain, R_{Ib}, retained the acquired resistance even after it was subcultured in absence of copper. This usually holds true even for ten or more passages through the normal medium. The survival ratio of $R_{Ib(O)}$ was, however, a little lower than that of R_{Ib} when the copper concentration was higher than 3 mM. It is conceivable that

Fig. 1. Survival ratio-copper concentration relation of the parent strain (A), R_{Ib} (B) and $R_{Ib(O)}$ (C).

the conversion from R_{Ib} to $R_{Ib(O)}$ and the reverse may be a de-adaptation and a re-adaptation which occur readily. When high copper concentrations are used, some cells of the de-adapted population, $R_{Ib(O)}$, might be injured before they, or their immediate descendants, became re-adapted, the result being a slight lowering of the survival ratio.

The colonial growth was observed by surface plating. When an order of 10^6 cells of the parent strain were plated on 2 mM Cu-MH agar medium, there appeared an order of hundred irregularly shaped colonies composed of white papillae, among which brown and light brown papillae were found. The distribution of the size of colony, as examined after one week's incubation, ranged from the largest to those which were hardly visible to the naked eye. On surveying the plate surface with microscope, there was a continuous gradation of colonial size down to a single cell. Among visible colonies, there were a few large colonies carrying white, light brown or brown sectors. Only in rare cases the R_{Ib}-type colony was found. This type of colony may have originated from a cell of R_{Ib}-type, or else from a cell of the parent-type if the R_{Ib}-type cell was formed in the clone in so early a period that cells less resistant than R_{Ib} were overgrown and hence the sector was not formed.

When parent cells were plated on the surface of copper agar, the counting of "visible" or "large" colonies was difficult, since the gradation of colonial size was continuous. However, a rough estimation was made by arbitrarily deciding the visible limit of colonies. According to this result, the survival ratio by the surface
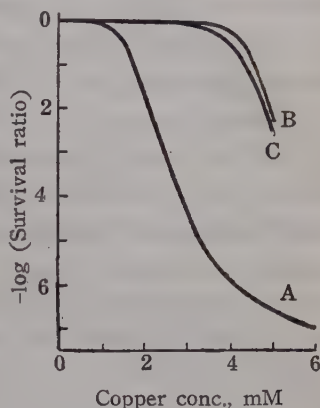
plating method was higher than that by the pour plating method.

It has become evident from the surface plating experiment that the number of colonies which appear on and in the copper-agar medium does not necessarily represent the number of copper resistant cells which were present in the original culture, but represents in most, if not all, cases the number of cells which could survive and grow to be visible. The colonial growth seems to be accompanied by the appearance of cells endowed with certain degrees of resistance, brown papillae growing when R_{Ib} cells were produced.

Training culture

One m*l* of the parent strain culture in its stationary phase of growth was pipetted into 100 m*l* of the normal and the 1 mM Cu–MH media, and cells were counted using hematimeter at intervals during incubation. Fink's methylene blue was used to discriminate " dead " cells.
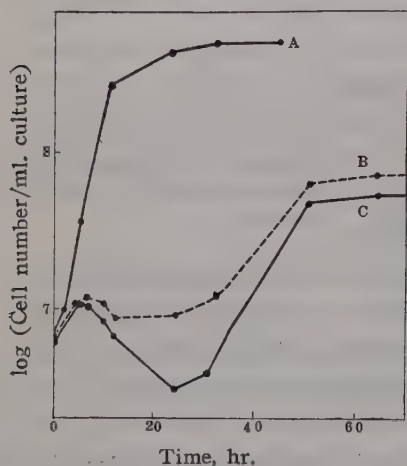
Fig. 2. Growth in liquid media. A: parent strain in MH; B: parent strain in 1 mM Cu–MH, total cells; C: the same, living cells.

The growth in the normal medium proceeded as shown by the curve A, Figure 2. In the copper medium, on the other hand, " living " cells increased a little at first, and then decreased slightly until the secondary growth took place after 30 hours or so, as represented by the curve C in the figure. Yanagishima *et al.*[2] reported a similar course of growth which occurred on the copper plate, and described the first logarithmic, the second lag, and the second logarithmic phases. The first logarithmic growth is usually smaller in the liquid medium than on the surface of the plate, when the copper concentration is the same.

The second logarithmic phase is due chiefly to the growth of resistant cells, because cells sampled from the culture in this phase can give rise to the R_{Ib}-type colonies on the 2 mM Cu–MH plate. Naiki *et al.*[3] found that the copper content of cells increased during the secondary growth, and Minagawa[4] reported that the R_{Ib}-specific ribonucleic acid became detectable when the second logarithmic phase began. All these findings show that resistant cells are predominating in the second logarithmic phase.

The secondary growth in copper medium ceases at a lower cell concentration than that attainable by the growth in the normal medium. The medium may have changed so much as to stop the rapid growth, since many surviving cells of the parent type, too, must have continued their metabolism. When cells at the final stationary phase are inoculated in a fresh 1 mM Cu medium, they reach the stationary population almost as high as in the curve A, although the time needed to reach

that level is usually a little longer than in the non-toxic medium.

When the parent strain was inoculated in 0.2 mM Cu liquid medium, the growth was monophasic. The growth rate and the stationary phase level were a little lower than in the normal medium. When the cells grown in this way were seeded on the 1.2 mM copper plate, the result differed from that by the parent strain only in that the relative number of very small colonies was smaller. However, no difference was found between the two when they were plated on media of copper concentrations higher than 1.5 mM. The content of 0.2 mM Cu in the medium may have effected only in eliminating very weak cells.

With the 0.7 mM copper medium, the decrease of cell number in the second lag phase was less conspicuous than with 1.0 mM. The strain trained in 0.7 mM copper medium showed lower resistance than R_{Ib}, only when the test plate contained copper more concentrated than 2 mM.

It is important to see how the nature of cells and the populational make-up change before the second logarithmic phase begins. One ml of the stationary phase culture of the parent strain was inoculated in the liquid MH medium containing 1.2 mM of copper. And samples were taken from this culture at intervals to be seeded on 2 and 1.2 mM copper plates as well as on the control plates.

If many of the parent cells inoculated in the copper medium should change gradually to R_{Ib}-type in the second lag period, the cells sampled at a later part of the period would grow into R_{Ib}-type colonies on the copper plate, with less irregularities in the colonial form than those sampled earlier. If, on the other hand, the second logarithmic growth was a continuation of the growth of a small number of resistant cells, a number of regular R_{Ib}-type colonies would grow even from samples taken at early stages in the second lag phase. As mentioned in the foregoing section, the appearance of an R_{Ib}-type colony may not necessarily mean that there had been an R_{Ib}-type cell among the cells plated. However, since the chance of the parent-type cell producing an R_{Ib}-type colony is very small, the plating method is reasonably adoptable for the present purpose.

A representative result is presented in Figure 3. The viable count on the control plate decreased up to 30 hours of incubation (curve A). The cell count by hematimeter showed that cells had already begun increasing by this time, as was shown in Figure 2. The viable count was, however, much smaller than the total cell number at this period, because many of the new-born cells were still attached to their mother cells. The mean cell number per cell-cluster was not less than 2 at this initial period of the (second) logarithmic phase.

The viable count on the 1.2 mM plate was always lower than that on the control plate (curve B, Figure 3). Notwithstanding that the cells had been in the 1.2 mM liquid medium, some of the "viable" cells failed to grow on the plate containing the same concentration of copper. It should be mentioned here that the copper toxicity becomes less when agar is added to the medium, perhaps because of interac-
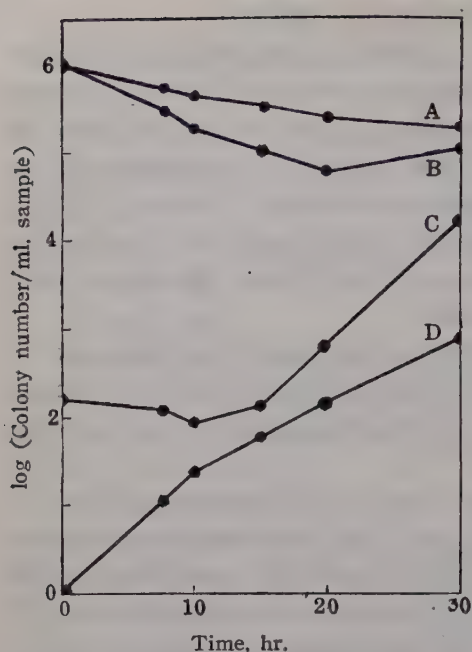
Fig. 3. Number of colonies grown on agar plates seeded with samples from 1.2 mM Cu–HM culture of the parent strain. A: On the control plate; B: on 1.2 mM Cu plate; C: on 2.0 mM Cu plate; D: R_{Ib}-type colonies on 2.0 mM Cu plate.

tion between copper and agar.

The curve C, Figure 3, represents the change of the number of all visible colonies growing on the 2 mM plate, and the curve D the R_{Ib}-type colonies among them. These were less than one per 10^6 plated cells at the start of the training culture, and increased roughly at a rate of one doubling every 2 to 4 hours. On the other hand, the increase of the total colonies occurred after 15 hours. This was chiefly due to the increase in number of large irregular-shaped colonies, presumably originating from cells with a little higher resistance than the majority of the parent population.

Thus it is evident that cells which are more resistant than the majority of the parent type survived and grew selectively during the second lag phase of the training culture. However, it can not be decided whether the rate of increase of resistant cells is higher than what would be expected from the simple multiplication, because the colony counting method only gives the number of cell-clusters, instead of that of individual cells. No definite conclusion could be drawn from the above result as to whether resistant cells are produced from sensitive ones during the second lag period.

Resistant cells in the parent strain

An attempt was made to determine whether the parent population contained cells with resistance heritable to their clonal offsprings.

The fluctuation test by Luria and Delbrück[5] could not be used, because the variance of the colony number was too large, even among the copper plates which were inoculated with samples from a suspension of parent cells.

The results obtained with Lederberg's replica plating method[6] were not so clear-cut as in his own example, since the sensitive cells had a very high chance of producing visible colonies in the present case. Calculations were made to see if the number of colonies developing on identical sites on each replicate plate was larger than that expected on the hypothesis that every colony on the master plate had an equal chance of growing on the replicate copper plates. Actually it was a little larger than the latter. However, there might have been some colonies of

which more cells were printed on every replicate plate than the others, by chance or because their size was large. Those colonies may have had a higher chance of growing on all of the replicate plates. So it can not necessarily be inferred by the above result that the parent population involved resistant clones in it.

The indirect selection method[6] was tried, for the purpose of selecting R_{Ib}-type cells from the parent culture. But repeated trials resulted in a failure. When, on the other hand, a number of $R_{Ib(O)}$ cells had been introduced in the parent culture, resistant cells could be selected by the same procedure. However, the indirect selection became more and more difficult as the proportion of $R_{Ib(O)}$ cells mixed in the parent cells was lowered. This may be due to the fact that R_{Ib}-type cells are overgrown by parent cells in the normal medium. Thus the negative result did not necessarily mean that the parent population did not involve cells which carried heritable resistance.

Newcomb's spreading experiment[7] was modified as follows. Filter paper was impregnated with MH–agar medium. The parent cells were spread on it. After a suitable period of incubation one group of the filter paper was transferred on 2 mM Cu–MH plates, the paper being inverted so as to place the micro-colonies in direct contact with the copper medium (the unspread plate). For preparing the

Table 1. Resistant colonies developing from microcolonies in contact with copper, with and without previous respreading.

Incubation. (hr.)	18	22	25.5
Cluster plated	369	369	726
End No. cluster	1.5×10^7	6.9×10^7	3.7×10^8
Factor increase	4.0×10^4	1.9×10^5	5.2×10^5
Resisistant colonies on			
Spread plate	12.5	50.5	80
Unspread plate	8.7	18.7	44
Ratio Spread : Unspread.	1.4	2.7	1.8

spread plate, the micro-colonies on the surface of agar paper were redistributed by glass rod before they were brought into contact with the copper plate. After incubation, large brown colonies were counted, observed through the agar plate. Average counts per plate are given in Table 1.

If the resistant colonies were of the clonal origin, the ratio "spread : unspread" should become larger as the growth of micro-colonies proceeds with time. But actually the ratio remained as low as 2. This result makes it very doubtful that the parent strain carries in it clones which have a higher chance of thriving in copper medium than other clones. It, however, was not necessarily disproved, because resistant cells, once produced in a micro-colony, may have failed to proliferate competition with the parent cells. On the other hand, the fact that the ratio

"spread: unspread" was about 2 may be explained by supposing that the large brown colonies originated from the most proliferative of the plated parent cells, the cellular vigor (not the copper resistance in particular) being transmitted to daughter cells with some probability.

Experiments so far described are concerned with the ability of cells to grow on the copper medium. An experiment was designed to see if the relative susceptibility of cells to copper in non-nutrient medium is more uniform within a clone than among cells of different clones.

Parent cells were suspended in MH-agar, and this was solidified in sheets of 2 mm thickness. When many of the dispersed cells multiplied in the agar sheets, becoming to 4- to 8-celled micro-colonies, the sheets were shaken in $M/15$ KH_2PO_4 solutions for different periods, to injure the cells to various degrees. After being washed with KH_2PO_4 solution again, the agar sheets were soaked in Lindegren's methylene blue solution. Stained cells were counted as dead.

The proportion of dead cells in each sheet was determined and the number of micro-colonies in which the constituent cells were all dead or all living was separately counted. On the assumption that each cell has an equal chance of being killed by copper, the probable frequencies of the totally dead and the totally alive micro-colonieswere calculated. The calculated and the observed values are given in Table 2.

Table 2. Counts of the totally alive and totally dead colonies.

No. of cells per colony	No. of colonies observed	Proportion of dead cells	No. of totally dead colonies		No. of totally alive colonies	
			Calc.	Obs.	Calc.	Obs.
8	32	0.875	11	22	0.0	0
8	37	0.622	1.4	5	0.0	0
8	42	0.464	0.09	5	0.3	5
4	73	0.384	1.6	4	10.5	32
4	73	0.187	0.09	2	32	47
4	90	0.150	0.04	2	47	60

Except when no totally alive colonies were found because of heavy injury, the observed numbers of totally dead and totally alive colonies were a little, but with statistical significances, larger than the respective calculated numbers. Hence there is a tendency that the relative ease with which a cell is killed by copper is transmitted to its clone at least for two or three generations. The tendency became obscure when one more generation was passed, though the observed samples were not many. It should be noted that the number of totally dead and totally alive colonies was not so many as to permit the presumption that the copper-susceptibility is a character transmitted to descendant cells with a high probability.

Although the growth competition between the resistant and the present-type cells does not come into the question in this experiment, the following should still be born in mind. 1) The observed cells were probably not of R_{1b}-type, because the cells of this type, even if present in the parent population, must be less than

$1: 10^6$. Hence the experiment has probably dealt with the order of statistical variation of reistance in a simple population. 2) The ability to proliferate in the copper nutrient medium is not necessarily correlated with the ability of surviving in copper medium under the non-growing condition.

Microscopic tracing of clonal growth

When cells of the parent strain are spread on the 1.2 mM Cu-MH plate, more than 90% of the seeded viable cells grow into visible colonies, including very small ones. Most of them carry a number of small white papillae, brown papillae also appearing on many colonies sooner or later. The brown papillae are composed of cells which grow into R_{1b}-type colonies when transferred to fresh copper agar plates. Hence the parent cell has a very high probability of producing R_{1b}-type cells in its clone growing on the copper medium as papillae, whereas the probability of producing the R_{1b}-type colony itself is extremely small.

Hence the development of parent cells on the copper plate and the production of papillae therefrom was followed using a microscope. A slide glass with a hole, 1 cm in diameter, was placed on another ordinary one. The well thus formed was half filled with 1.2 mM Cu-MH agar, and parent cells were spread on its surface. A cover slip was placed to cover the hole, and was fixed with dots of paraffin to keep the inside aerobic. In this preparation, each cell and the micro-colony growing from it could be recorded and identified during the whole period of development, by the combined use of mechanical stage and checkered ocular micrometer.

Many of the seeded cells budded at first, but the budding decreased gradually. Few cells produced more than three daughter cells. The newly produced cells also lost the budding activity with time. Perhaps they were injured by copper which was penetrating them. Only the young cells, and even only a limited number of them, produced buds. So the growth rate of micro-colonies became very small*, they taking irregular ramified forms.

Methylene blue disclosed that most of old cells were dead. The number of living cells decreased, as shown in the curve C, Figure 2, the death rate** perhaps surpassing the growth rate as referred to living cells. Colonies stopped growing when none of their young cells produced new buds, thus leaving dead colonies of various microscopic sizes.

Many micro-colonies, however, continued their very slow growth. Then, in one of these micro-colonies, a group of cells was found in which cells did not lose the budding activity so fast as before. This cell group grew rapidly, since most of the cells continued budding. It grew forming a smooth round outline, to develop into a papilla later. It was difficult to determine whether the " generation time "*** of this

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* The growth rate was very small even when it was referred to the living cells, instead of total (living and dead) cells.

** Number of death per unit time, per living cell.

*** Either the period from the birth of a cell to the bud formation by this cell, or the period from the formation of a bud to that of the next bud by a cdll.

new type of cells was shorter than that of the original type, but it seemed certain that the larger rate of growth in the new type of cells was mainly due to their longevity.

It was thus observed how papillae originated in a micro-colony growing from a parent-type cell. However, since the plate was thin and the colony density was high, the papillae did not grow large enough to be differentiated in color, white or brown. So, it may not be justified to state that the development of brown papillae was actually observed under microscope among multitudes of white ones.

When cells of white papillae were spread on copper agar medium, percentage occurrence of large colonies was higher than when parent cells were seeded. But the colonies were mostly irregular in shape.

Hence, the cells of white papillae are considered to be not fully but intermediately resistant. However, the resistance of such cells was not stable enough to permit more precise studies. It is not yet known either whether the R_{Ib}-type cells are produced solely from cells having an intermediate resistance, or whether the direct change from the parent-type to the R_{Ib}-type is possible.

The microscopic observation reported above has shown the clonal basis of the gross phenomena represented by the curve C, Figure 2, and the curves C and D, Figure 3. However, there may be some difference in the growth process according to whether the medium is solid or liquid. One point to be mentioned is that the clones of inoculated cells have more chance of producing independent resistant clone in the solid medium than in the liquid one, because, in the latter, the resistant cells which are formed early may multiply so much as to leave no room for later formation of resistant cells in other clones.

Discussion

Various types of colonies grow from the parent strain plated on 1 mM Cu–MH agar. When cells of these colonies are transferred to fresh copper media, they grow with various degrees of ease, but more easily than the parent cells. Among such varient cells, the R_{Ib}-type has a tendency to overgrow the other in 1 mM Cu-MH medium. So the substrain, R_{Ib}, is easily established and sustained in the copper medium. It is stable also for a considerable number of subcultures in non-copper media. Hence R_{Ib} is chiefly used at present for studies of copper resistance.

The genetical nature of R_{Ib} is not yet quite clear. Results of tetrad analyses of hybrids between R_{Ib} and its parent strain, and those between R_{Ib} of a strain[10] and other untrained strains,[9] were more complex than in the case[10] where an untrained resistant strain was crossed with other sensitive strains.

Let R_{Ib} be assumed a mutant. Then, copper may appear to be essential in causing the mutation, since the colony grown from a parent cell has a very high chance of producing papillae of R_{Ib} cells, in spite of that the presence of R_{Ib}

cells can not be proved in the parent population. But the spontaneous mutation and selection hypothesis can also hold: The presence of cells with intermediate degrees of resistance suggests the multistep pattern of the resistance. R_{Ib} may be established through repetition of selection of a more resistant mutant, followed by further spontaneous mutation of the selected. In this case, the chance of an R_{Ib} cell being found in the parent population should naturally be extremely low.

However, even if the multistep idea is adopted, a possibility still remains that copper is effective, or even essential, in some steps of mutation. A possibility is not rejected either, that the one-step mutation from the parent type to R_{Ib} occurs in the copper medium.

If, on the other hand, R_{Ib} is assumed to arise solely through phenomic adaptation, the intracellular factor (or condition) which governs the mechanism(s) of copper resistance should be permanently modified during a single passage through the copper medium. The present case differs from those of Oxford authors[11] who found that repeated training was necessary to stabilize the acquired resistance.

It was observed under microscope that, in copper media, the growth rate as referred to the number of viable cells increased when cells were produced which did not lose budding ability so soon as sensitive cells. By unpublished experiments it was found that R_{Ib} was sensitive to copper, even as sensitive as the parent strain in some cases, when the medium was deficient in any one ingredient of the minimal synthetic medium. Studies on the resistance mechanism, which was partly suggested by Naiki *et al.*[3], will be reported elsewhere.

Summary

1. The substrain, R_{Ib}, obtainable by training the parent strain on 1 mM copper medium, forms round and smooth brown (viz. the R_{Ib}-type) colonies on 1–2 mM copper agar. On this medium, the parent strain grows irregular colonies carrying white and brown papillae. The latter papillae contain cells of the R_{Ib}-type.

2. Even after serial subcultures in the normal medium, R_{Ib} forms the R_{Ib}-type colonies on the copper medium. However, a little deadaptation was disclosed when the resistance test was made with higher concentrations of copper.

3. Selective growth of cells of intermediate resistance, as well as of R_{Ib}-type, as observed when the parent strain was inoculated in a liquid copper medium.

4. The clonal occurrence of resistance could not be observed in the parent strain.

5. Many of the clones of parent cells spread on 1.2 mM copper agar can continue to grow very slowly, older cells losing their budding ability almost as rapidly as new ones are produced. When cells are formed which do not lose the budding ability so soon, the group of such cells grows rapidly and forms a papilla.

Acknowledgments

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Dorsiventral Structure of Unifacial Leaves
in Several *Iris* Species

Shun-ichiro IMAMURA* and Michiko HIDA**

今村駿一郎* ・ 肥田美知子**：　二三のアヤメ属植物における単面葉の背腹構造

In some plants the dorsiventrality of leaves, expressed in the distribution of stomata and assimilating parenchyma, is determined by their situation with respect to the direction of external factors. Some conifers, *Thujopsis dolabrata, Chamaecyparis obtusa, Podocarpus imbricata*, etc. (3, 4, 5, 6, 7, 9, 11), have assimilating organs, whose dorsiventrality is determined by the direction of incident light. Gravity as a determining factor was reported previously by the senior writer in *Iris japonica* (8). Dorsiventrality can be readily recognized from the different color of the upper and lower leaf surfaces. It is rarely found in *Iris* species having equitant leaves, which usually have an isobilateral structure. But such cases of dorsiventrality were later found in 5 species; they are described in the present paper.

* Laboratory of Applied Botany, Faculty of Agriculture, Kyoto University, Kyoto, Japan.　京都大学農学部応用植物学研究室
** Biological Laboratory of Osaka Women's University, Osaka, Japan.　大阪女子大学生物学教室

I. Anatomical observations

1) *Iris formosana* Ohwi.

The species was first collected by Dr. Jisaburo Ohwi in Formosa in 1934 (12). It is similar to *Iris japonica* but is larger in size, and it bears fewer flowers on an inflorescence and is fertile.* The upper surface of the leaf is dark green whereas the lower surface is waxy and glaucous. The collateral fibrovascular bundles do not occur in pairs but occur alternately. In the distribution of the conductive strands no dorsiventrality was found. Only a very few stomata are found on the upper surface, but many short cells occur here among the longer epidermal cells (Plate XIX, Fig. 5a). As previously reported (8, 10), the short cells are rudiments of stomata. The cell layer beneath the epidermis of the upper side has relatively small intercellular spaces. On the lower surface many stomata and a small number of short cells are found (Plate XIX, Fig. 5b). The subepidermal cells are cocoon-shaped with large intercellular spaces between them.

2) *Iris Rossii* Baker.

For this material we are indebted to Mr. Taketo Mizoguchi, who collected the plant near Beppu in North Kyushu.

Fig. 1. Transverse sections of *Iris* leaves, anatomical upper surface shaded, × unpaired vascular bundles. A: *Iris Rossii*. B: *Iris uniflora*. C: *Iris Wilsoni*. D: *Iris Bulleyana*. A and B × ca. 50. B and C × ca. 40.

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* *Iris japonica* has 54 chromosomes in somatic cells and, being a triploid, does not set seeds. A very similar plant was collected by Yu, Ching-jang in Central China, which had 36 chromosomes in somatic cells (17). The leaf structure coincided entirely with that of *Iris japonica*.

The leaf is thin and is strongly striated. The species is similar to *Iris japonica* and *formosana* in the arrangement of the conductive strands which do not occur in pairs but occur alternately; no air channels are found in the mesophyll (Fig. 1, A). The epidermis of both sides is very wavy (Plate XIX, Fig. 1a). Stomata are almost entirely restricted to the lower surface while on the upper surface a small number of short cells are found (Plate XIX, Fig. 1b).

3) *Iris uniflora* Pall.*

For this material we are indebted to Mr. Ryuhon Saito who kindly collected the plant upon our request in middle Korea. In a cross section of the leaf, major collateral fibrovascular bundles stand in pairs, between which minor unpaired bundles are found on the upper side (Fig. 1, B). Between the large vascular bundles air channels are formed. The upper leaf surface is dark green and has scarcely any stomata (Plate XIX, Fig. 2a). In contrast to *Iris formosana* and *Iris japonica*, short cells are scarce on the upper surface. The lower leaf surface is glaucous and has many stomata (Plate XIX, Fig. 2b). The subepidermal layer is rich in intercellular spaces.

4) *Iris Wilsoni* Wright and 5) *Iris Bulleyana* Dykes.

These two are species from south-western China. We obtained herbarium material and viable seeds by the courtesy of Dr. M. Reed, Brooklyn Botanic Garden, U.S.A.

The two species are very similar in their anatomical leaf features. The distribution of the conductive strands is similar to that of *Iris uniflora*, having paired vascular bundles and a small number of unpaired ones on the upper side (Fig. 1, C, D). The stomata are restricted almost entirely to the lower surface. Rare short cells are found on both surfaces (Plate XIX, Fig. 3a, b; Fig. 4a, b).

With the exception of *Iris japonica* and *Iris Rossii* all species have papillae on the epidermal cells, which are especially conspicuous on the lower surface (Plate XIX, Fig. 2b, 3a, 3b, 4b and 5c).

II. Physiological observations

Observations and experiments were performed to investigate the factors which induce the dorsiventrality of leaves. Mainly, *Iris japonica*, *Iris formosana* and *Iris uniflora* were used as materials. In the mode of dorsiventrality induction two types could be distinguished.

a) Labile induction

The dorsiventrality of a newly developing leaf can be easily inverted by the reversal of the direction of the controlling external factors. In a vertical position the developing leaf becomes isobilateral with stomata on both surfaces. When the plant is fixed in an inclined position the stomata and spongy parenchyma develop on the surface directed downward whereas on the opposite side many short cells

* Synonym. *Iris ruthenica* Ker-Gawl. var. *nana* Maxim.

and few stomata are formed. By repeated reversals we can obtain plants, whose leaves have a reversed dorsiventrality. Seen from one side, some leaves appear dark green while others are pale green. A leaf, which has areas with and without stomata side by side on one surface, can also be obtained. *Iris formosana* and *Iris japonica* belong to this type. The inducing factor is gravity, and light, so far as the experiments indicate, has no significant rôle in induction (8). One experiment with *Iris formosana* will be mentioned here.

Vigorous plants with developing leaves were transferred to a dark room and
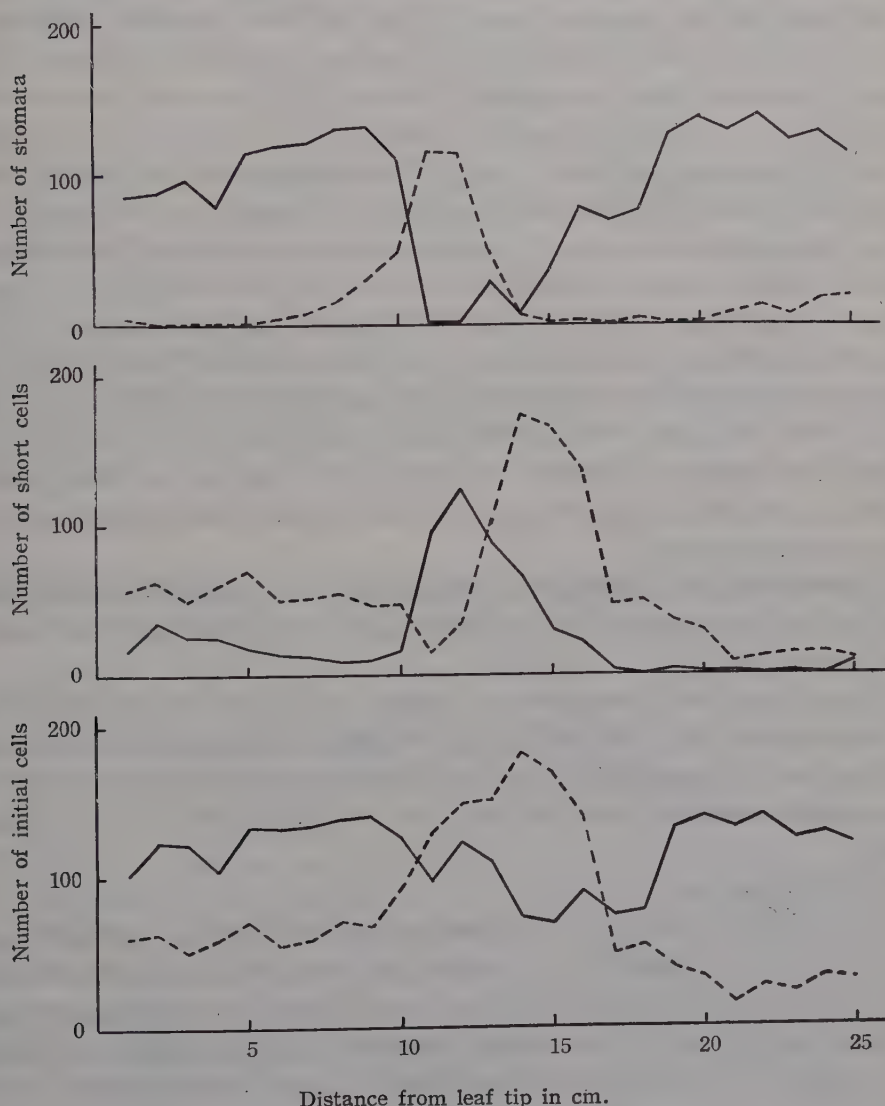

Fig. 2. Distribution of stomata, short cells and initial cells in a leaf, which was fixed in inverted position for 54 hours while developing in the dark room. *Iris formosana*. Solid line initial upper surface, broken line lower surface.

kept in an inverted position by fixing the basis of the plant with metal forks. After having been left in the dark for 54 hours they were returned to the initial position and grown in day light. When the developing leaves had attained their full size, the distribution of stomata and short cells was observed and the average size of the epidermal cells was established from the tip to the base in intervals of 1 cm. The observations carried out on one plant are represented in Fig. 2. The initial length of the leaf was 13.7 cm and it became 15.8 cm in the dark room. The final length was 44.7 cm and the leaf blade was somewhat constricted in the middle portion, indicating that the expanding growth of the leaf was somewhat inhibited by darkness. As the ultimate density of cells per unit area may be modified by the subsequent expanding growth, the number of stomata and short cells observed were corrected by the size of the surrounding epidermal cells to make clear the mode of differentiation in young epidermal tissue.* The distribution of the stomata shows a completely inversed dorsiventrality in a portion between 10 and 13 cm from the leaf tip. The number of stomata is strikingly reduced on the initial lower surface, which was directed upward in darkness and markedly increased on the opposite surface. On the other hand, the short cells increased on the former surface and decreased on the latter. In the transition zone various types of intermediate structures of stomata and short cells are found, indicating the homologous nature of both cell types (10). An initial cell can differentiate into a short cell or two guard cells, according to its direction in relation to gravity. The initiation of initial cells is influenced by the action of gravity, and they are formed in larger numbers on the surface directed downward.

The inversion in the distribution of initial cells on the experimental leaf reaches nearer the leaf basis than that of stomata (Fig. 2). The behavior is the same as in *Iris japonica* (8).

The seedlings of *Iris formosana* are prostrate and gravity acts only in one direction. Several first leaves are slightly dorsiventral, having more stomata on the lower than on the upper surface. In the following leaves the dorsiventral character is gradually intensified up to the 4th or the 5th leaf, in which final structure is attained.

b) Stable induction

To this second group belong *Iris uniflora* and *Iris Rossii*. In this group, once the dorsiventrality is established, it cannont be influenced by experimental treatment. Not only the developing leaf but all leaves, which later develop, retain the same dorsiventrality as the older leaves, irrespective of the inclination of the plant to the vertical line. Whether the lateral shoots of higher order which may develop

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* The relative size of epidermal cells was determined, taking their average size on the lower side at the leaf tip as a unit. The number of stomata and short cells at each point of Fig. 2 was obtained from the observed number multiplied by the relative dimension of the epidermal cells.

long after the fixation in inverted position, may also retain the same dorsiventrality as the mother axis, could not be decided, as our experiments lasted only about a half year.

Table 1.   Number of stomata per mm² in seedling leaves of *Iris uniflora*, average of ten observations.

Plant	Surface	Main axis	Axillary shoot of the		
			first leaf	second leaf	third leaf
1	I	157±4.6	173±11.9	17±3.8	111±8.6
	II	167±9.8	11± 3.9	122±6.7	22±3.7
2	I	186±6.0	192± 5.8	194±8.2	53±3.8
	II	184±7.0	126± 5.6	42±6.2	144±6.5

Leaves on the main axis of a seedling of *Iris uniflora* are erect and do not show any sign of unequality of both surfaces. The lateral branches, however, have distinctly dorsiventral leaves. Individual branches are not dorsiventral in the same sense, as shown in Table 1. The arrangement of leaves and shoots of a seedling is given diagrammatically in Fig. 3. In the early stages of development, the plane
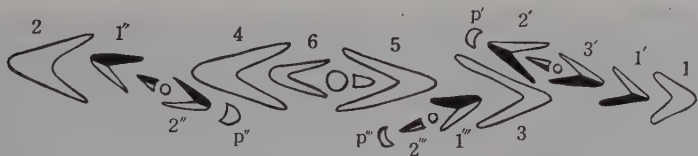
Fig. 3,   Diagram of branching in a seedling of *Iris uniflora*, anatomical upper surface solid black, p, prophyll.

of a lateral shoot is almost parallel but slightly oblique to that of the mother axis (13, 16). The leaf side of a lateral shoot which stands nearer to the mother axis, becomes the anatomical upper surface, and the opposite side becomes the lower surface. Induction occurs, even if the basis of the plant is deeply covered by soil at a very early stage of germination. As the determining agency there is no reason to assume any external factor other than gravity. A slight inclination of the initials of the lateral shoots may induce dorsiventrality of the growing point from which the following leaves are developing. Also *Iris Bulleyana* and *Iris Wilsoni* do not show any dorsiventrality in the main axis of the seedlings. In all probability these two species belong to the second type of induction although we could not perform any detailed experiments.

Discussion and Summary

In all *Iris* species with unifacial leaves the rhizome lies in the soil horizontally and is distinctly dorsiventral. In most of them, the foliage shoots with equitant and isobilateral leaves which stand erect, both leaf surfaces being exposed equally to light and gravity. However, some species, for instance, *Iris gracilipes* A. Gray, *Iris tectorum* Maxim., etc. have plagiotropic shoots which do not show any anatomical unequality of the surfaces of their leaves.

In the species described in the present report a structural dorsiventrality was found. In *Iris formosana* the seedling has, except for the first 1 or 2, dorsiventral leaves. The dorsiventrality can be inverted by changing their direction in relation to gravity, as in *Iris japonica*. In *Iris uniflora* and *Iris Rossii* the seedling is not dorsiventral but the lateral shoots are distinctly so. Once established, this dorsiventrality cannot be inverted by changing the direction in respect to external factors. The growing point seems to be determined dorsiventrally and it gives rise to leaves with unequal surfaces. In this case also gravity seems to have the determining rôle. In the mode of induction *Iris Wilsoni* and *Iris Bulleyana* seem to belong to the stable type.

It is noteworthy that all the above species are members of Asiatic flora. They belong to the section *Apogon* except *Iris japonica* and *Iris formosana*, which are representatives of the section *Evansia*. The taxonomic or systematic significance of leaf dorsiventrality cannot be evaluated until more *Iris* species are thoroughly examined. In literature the indications are that many have leaves with unequal surfaces.*

The causal sequence of aitiogenous induction of dorsiventral differentiation of the leaves is yet obscure. The unequal distribution of food materials and some determining hormones may probably be the factors which cause the unequal differentiation, as can be assumed from the eccentric growth in the thickness of the inclined trunks or lateral branches in many plants (14, 15).

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* In "The Genus *Iris*" of Dykes, the remark, "Upper side glossy and lower side glaucous" is found in the description of leaves of *Iris Forrestii*, *I. Clarkei*, *I. bracteata* and *I. graminea* (2). That the assimilating parenchyma differs on both surfaces in *Iris Douglasiana* is shown in Fig. 9 of Plate 80 in "Monocotyledons" of Arber (1).

1a   1b   2a   2b

3a   3b   4a   4b

5a   5b   5c
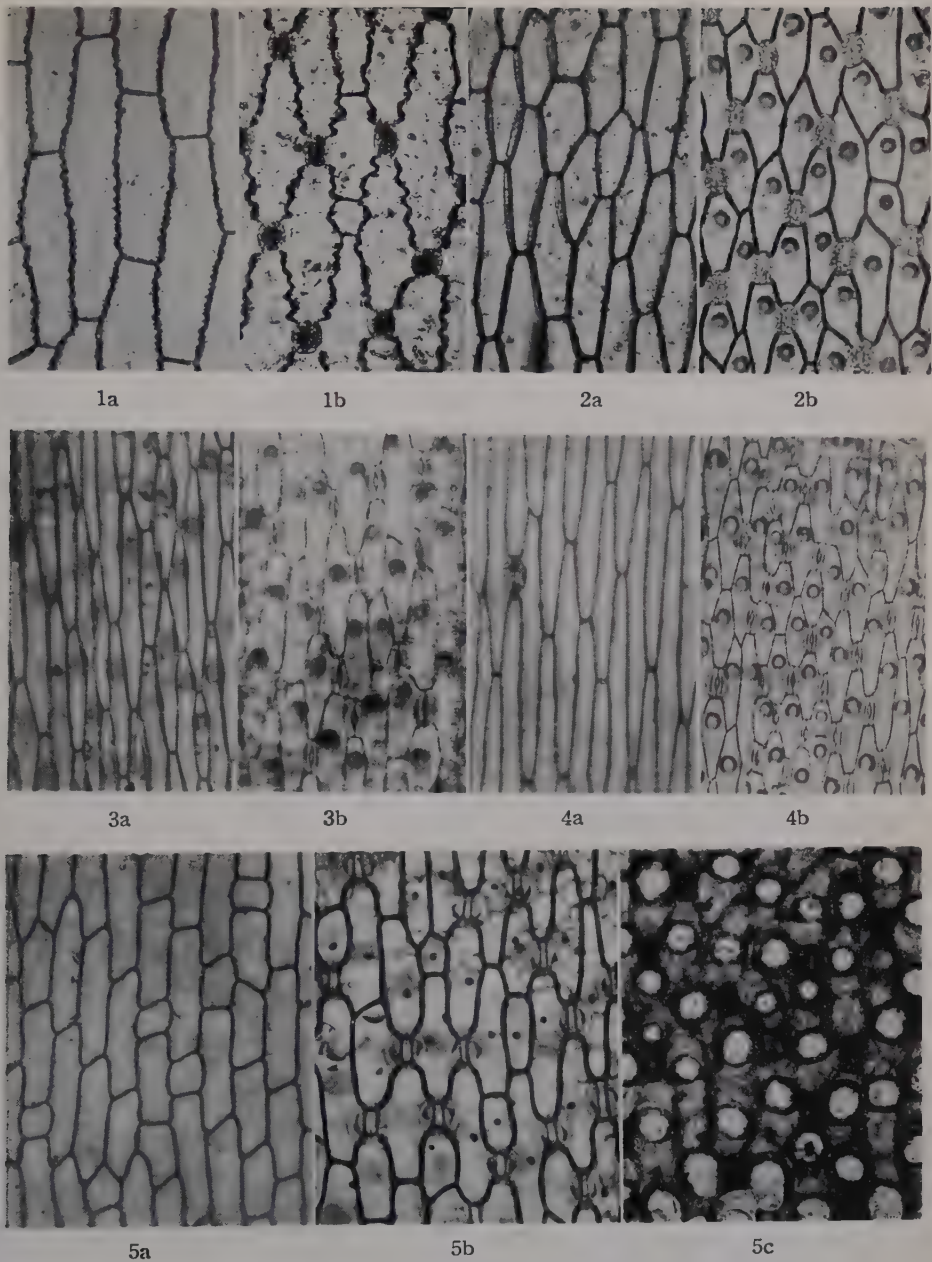
Plate XIX.   Epidermal tissues of *Iris* leaves.

Fig. 1.   *Iris Rossii.*
Fig. 2.   *Iris uniflora.*
Fig. 3.   *Iris Wilsoni,* from herbarium material.
Fig. 4.   *Iris Bulleyana,* from herbarium material.
Fig. 5.   *Iris formosana.*

a : upper surface,   b : lower surface,   c : lower surface, mounted with small quantity of water to make the papillae of epidermis clear. × ca. 150.

S. Imamura and M. Hida : Dorsiventral structure of unifacial leaves in several *Iris* species.

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Further Studies on Paper Chromatography of Anthocyanins, Involving an Examination of Glycoside Types by Partial Hydrolysis[*]

Studies on Anthocyanins XXIX[**]

by Yukihide ABE[***] and Kôzô HAYASHI[****]

阿部幸頴[***] ・ 林　孝三[****]：　アントシアン配糖体およびその部分的加水分解産物の
ペーパークロマトグラフィー（アントシアン色素の研究　第 29 報）

Since the technique of paper chromatography was first introduced by Bate-Smith (1, 2) into characterization of natural anthocyanins, remarkable progress has been made by succeeding investigations of several workers (3-6). Though modern procedure in these experimentations proved to be an effective tool for prompt determination of sugar-free pigments, yet something seemed to be still required for the correct determination of individual glycosides, especially of their glycoside types. In the course of our studies on paper chromatography of natural anthocyanins, we have found that a diglycoside in general is partially hydrolyzed to give monoglyco-

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side on treatment with acid under mild conditions and that the reaction process can be traced distinctly on the chromatogram.

On the other hand, the analysis of natural anthocyanins by means of paper chromatography, which was previously described by us (4), has been improved further by application of several new solvent mixtures.

Present paper deals with these subjects aiming at better application of the chromatographic technique necessary for the identification of natural pigments.

Our foregoing surveys on anthocyanins appearing in red autumnal leaves (7) and also in a number of flowers and fruits of alpine plants in Japan (8) were carried out throughout under application of the results descreibed herein.

Methods and Results

I. Characteristic behavior of several solvent mixtures

In order to obtain a sound basis for the identification of naturally occurring anthocyanins, further examinations were made using several solvent mixtures of excellent quality as shown in Table 1. In this experiment, following anthocyanidins and anthocyanins were used as standards: *Aglycone*—pelargonidin, cyanidin, peonidin, delphinidin, and malvidin; *3–monoside*—chrysanthemin, idaein, empetrin, and delphinidin 3–glucoside*; *3–bioside*—lycoricyanin and keracyanin; *3,5–dimonoside*—pelargonin, cyanin, peonin, malvin, and delphin*; *complex glycoside*—ensatin, shisonin, and nasunin.

Preparation of each original solution was made by dissolving a crystalline sample in cold 1% hydrochloric acid in a concentration of about 0.1%. Because of the sparing solubility, cyanin, delphin and 3–rhamnoglucoside of delphinidin were dissolved nearly to saturation by warming on a water bath. The cooled solution was spotted as usual on Tôyô No. 50 filter paper. Prior to irrigation, the paper was saturated with solvent vapor after standing for 24 hours in a chromatographic chamber. The chromatogram was developed all the time by one-dimensional ascending method at $25° \pm 2°C$.

Table 1. Solvent mixtures used in the present experiment
(Prepared at 25°C. and preserved for 24 hrs.
at the same temperature before use)

Designation	Composition (v/v)	Fraction used	Available for
Pro·H	iso-Propanol/5% HCl (55: 45)		Aglycone
Ac·H	Acetone/5% HCl (1: 1)		"
AA·H (5, 1, 5)	AcOH/36% HCl/H_2O (5: 1: 5)		"
AA·H (3: 1: 8)	" (3: 1: 8)		Glyc. & Agl.
isoA·H (21: 5: 4)	iso-AmOH/36% HCl/H_2O (21: 5: 4)	org. layer	"
Bu·A	n-BuOH/AcOH/H_2O (4: 1: 5)	"	Glycoside
Bu·H	n-BuOH/36% HCl/H_2O (7: 2: 5)	"	Glyc. & Agl.
Cr·A	m-Cresol/AcOH/H_2O (50: 2: 48)	"	Glycoside
Phen	Phenol/H_2O (9: 1 in wt.)		"

* Kindly sent by Sir R. Robinson.

As previously reported, the pigment spots on the chromatogram show more or less characterstic features with respects to coloration, fluorescence, and color reactions (cf. (4)), but a conclusive determination of pigment component cannot be achieved with these alone. However, fluorescence is in some cases quite useful, because 5-monoglucoside of pelargonidin, peonidin, and malvidin, which are produced by partial hydrolysis as stated below, are characterized by their conspicuous fluorescence under ultraviolet light, which is not the case with 3-monoglucosides of these anthocyanidins. The 3,5-diglucoside of pelargonidin (pelargonin) shows an intense yellow fluorescence on the chromatogram.

On the other hand, R_f value furnishes an effective index in the identification of individual pigments, and the following results should be referred to. The R_f values of pure samples of natural anthocyanins and anthocyanidins were carefully estimated using different kinds of solvent mixtures shown above (Table 1). The values, which are the mean of five or more replicates fluctuating only in a range of ±0.02–0.05, are plotted in Fig. 1, in which one can observe interesting relationships as follows: An increase in number of hydroxyl groups in the side benzene ring causes a considerable decrement of R_f value, while reverse is the case with methylation, as previouly stated by Bate-Smith *et al.* (2). As regards sugar attachment, increasing number of sugar residues results in a decrement of R_f values when developed with alcoholic as well as phenolic solvents, whereas the relation is quite reverse with aqueous solvents, *e.g.* AA·H(3, 1, 8), and formic acid–hydrochloric acid.*

For practical purposes, it may be useful to note here some properties of important solvent mixtures:

1. **Pro·H** (*iso*-propanol/5% hydrochloric acid)—This solvent is highly effective for the separation especially of pelargonidin and peonidin, which are scarcely distinguishable from each other with usual solvents. Shift of R_f value in accordance with the degree of hydroxylation and methylation as stated above is clearly demonstrated with this solvent.

2. **Ac·H** (acetone/5% hydrochloric acid)—Good for distinction between peonidin and malvidin, but sometimes, an appreciable fluctuation in R_f values may be encountered owing probably to the volatility of the organic component of this solvent system.

3. **AA·H(5, 1, 5)** (acetic acid/hydrochloric acid/water)—Distinct separation of pigments including both anthocyanidins and anthocyanins can be achieved with this solvent; especially good for aglycones in general. Degree of resolution is quite similar to that of the Forestal solvent (AcOH/conc. HCl/H_2O, 30 : 3 : 10 in vol.) of Bate-Smith (3).

4. **AA·H(3, 1, 8)** (acetic acid/hydrochloric acid/water)—While the resolution by this solvent is almost the same as that of the above, this seems to be better for

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* 80% HCOOH/36% HCl/H_2O (5 : 1 : 4, v/v) by T. Endô [Handbook of Biological Experiments, VIC, 63 (1954)].

Fig. 1. Graphic representation of R$_f$ values of anthocyanins and anthocyanidins showing
their characteristic behaviors towards various kinds of solvent mixtures
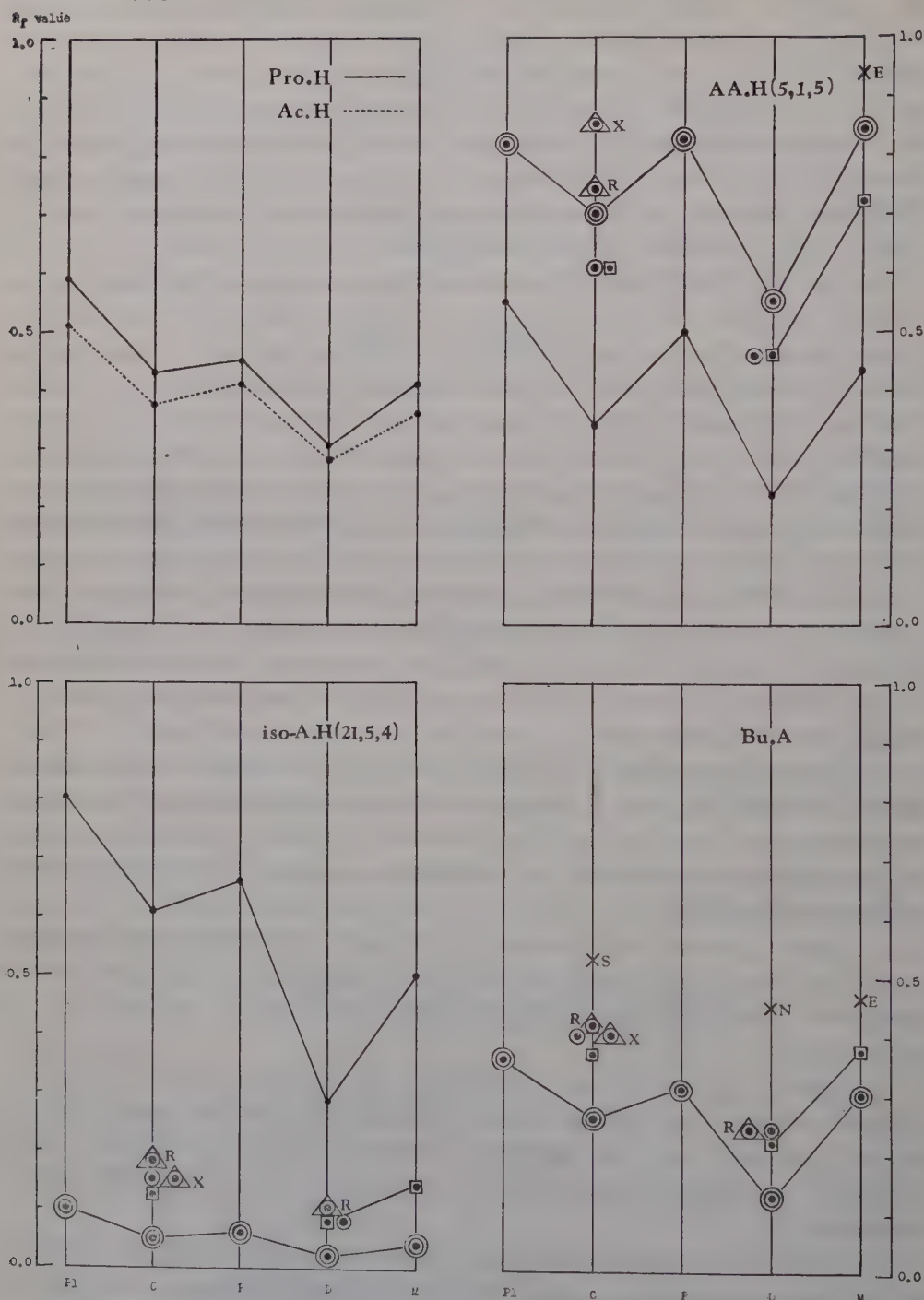
Abbreviations: Pl pelargonidin, C cyanidin, P peonidin, D delphinidin, M malvidin;
• aglycone, ⦿ 3-monoglucoside. ▣ 3-monogalactoside, ▲X 3-glucoxyloside, ▲R 3-
glucorhamnoside,◎ 3, 5-diglucoside.
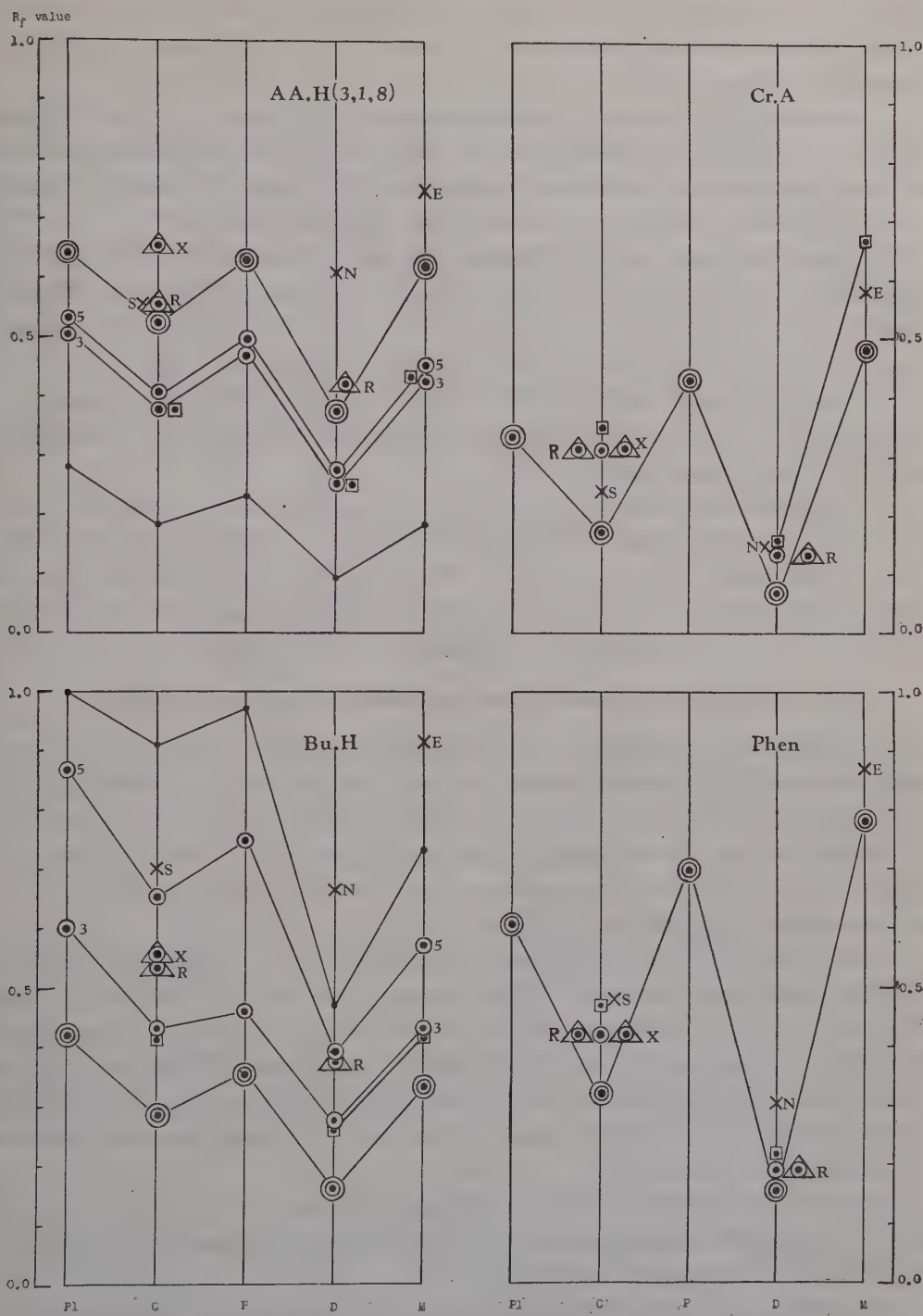
Abbreviations (continued): S shisonin (*p*-hydroxycinnamoyl-cyanin), N nasunin (*p*-hydro-oxycinnamoyl derivative of delphinidin 3-diglucoside), E ensatin (*p*-hydroxycinnamoyl derivative of malvidin 3-diglucoside), ⊙3 3-monoglucoside obtained by partial hydrolsis, ⊙5 corresponding product of partial hydrolysis; Pro.H, AA.H(5,1,8), etc.: see Table 1.

the study of glycosides, because its lower range of R_f values is convenient for practical use. This is available also for the discrimination of anthocyanins belonging to 3-rhamnoglucosides.

5. **isoA·H(21, 5, 4)** (*iso*-amyl alcohol/hydrochloric acid/water)—Degree of hydroxylation in sugar-free pigments as well as number of sugar residues attached to it are clearly indicated by this solvent, although the R_f values of glycosides are usually small and the spots of aglycones become more or less elongated.

6. **Bu·A** (butanol/acetic acid/water)—This solvent has the same composition as that described by Bate-Smith (1, 2). It distinctly resolves the degree of hydroxylation and glycosidation as well, giving clear-cut and compact spots of glycosides, whereas spots of the aglycones are apt to undergo decolorization.

7. **Bu·H** (butanol/hyrochloric acid/water)—Clear-cut results are obtained by this solvent in the analysis of some products of partial hydrolysis, when applied in parallel with the solvent AA·H (3, 1, 8).

8. **Phen** and **Cr·A** (phenolic solvents)—Presence or absence of methoxyl group is clearly indicated by these solvents, as demonstrated by Bate-Smith (2) in the case of cresol/acetic acid/water (50 : 2 : 48). Besides, it is noteworthy that in cyanidin derivatives the R_f value of 3-monoglucoside is lower than that of the corresponding galactoside, and reverse is the case with Bu·H ann Bu·A.

II. Paper chromatographic test on products of partial hydrolysis

A solution of a glycoside in 1% methanolic hydrochloric acid was mixed with equal volume of 20% aqueous hydrochloric acid and heated on a water bath at 70° C. During hydrolysis a small portion of the reaction mixture was sampled out at regular intervals and was spotted in duplication on a large sheet of chromatographic paper. Then the chromatograms were developed with different kinds of solvent mixtures, *e.g.* Bu·H and AA·H (3, 1, 8).

The experiments showed that the hydrolysis proceeded somewhat slowly and was completed after about two hours. Whole process of the reaction is shown in Table 2 and Fig. 2, in which following samples are included: 3-glucoside of cyanidin and delphinidin, 3–galactoside of cyanidin, delphinidin, and malvidin (Table 2 a); 3-gluco-xyloside and 3-glucorhamnoside of cyanidin, and glucorhamnoside of delphinidin (tulipanin*) (Table 2 b); 3,5-diglucoside of pelargonidin, cyanidin, peonidin, delphinidin, and malvidin (Table 2 c).

That the middle two of the four anthocyanin spots in Fig. 2, namely No. 2 and No. 3 from the top, are nothing but *5-monoside* and *3-monoside*, respectively, is confirmed by the following experiments:

(a) During the course of mild hydrolysis of dimonoside, four anthocyanin spots appeared in general; among them, spots No. 1 and 4 (Fig. 2 b) proved to be those

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* This new glycoside was isolated by Dr. M. Shibata (Toyama University) from the petals of a tulip variety (" Queen of the Night "). [Bot. Mag. Tokyo. **69**: 462 (1956)]

of aglycone and unchanged glycoside, respectively, by comparison of these with authentic specimens by reextraction and chromatographic treatment of the former.

(b) The spot No. 3 was ascribed unequivocally to that of 3-monoside, because the authentic specimen of the corresponding glycoside gave quite identical spots as regards R_f value and color reactions on the chromatogram. In fact, this was shown by careful comparison of the spot produced from either cyanin (3,5-diglucoside of cyanidin) or delphin (3,5-diglucoside of delphinidin) by partial hydrolysis with that of the corresponding 3-monoside, namely chrysanthemin and delphinidin 3-monoside. Accordingly, it follows that the remaining spot No. 2 should belong to a product having 5-monoside structure. Because of the absence of standard sample available for comparison, further proof could not be obtained.

(c) The fact stated above is clearly demonstrated in the case of pelargonin, *i.e.* 3,5-dimonoside of pelargonidin. On mild hydrolysis it gave four pigment spots

Table 2.   Pigment spots produced during partial hydrolysis of glycosides

a)   3-Monoside

Duration of hydrolysis (min.)	3-Mono-hexoside (original)	Aglycone
20	+++++	±
40	+++	++
60	++	+++
90	±	++++
120–150	—	+++++

b)   3-Bioside (glucopentoside)

Duration of hydrolysis (min.)	3-Gluco-Pentoside (original)	3-Mono-glucoside	Aglycone
10	++++	+	
20	++	+++	±
40	+	++++	+
60	±	++	+++
90	—	±	++++
120–150	—	—	+++++

c)   3, 5-Dimonoside

Duration of hydrolysis (min.)	Diglucoside (original)	3-Mono-glucoside	5-Mono-glucoside	Aglycone
20	+++++	±	±	—
40	+++	±	±	±
60	++	+	+	+
90	+	+	+	++
120–150	—	—	—	+++++

Fig. 2. Paper chromatograms of the products of partial hydrolysis
(a) Developed with solvent AA·H(3, 1, 8)
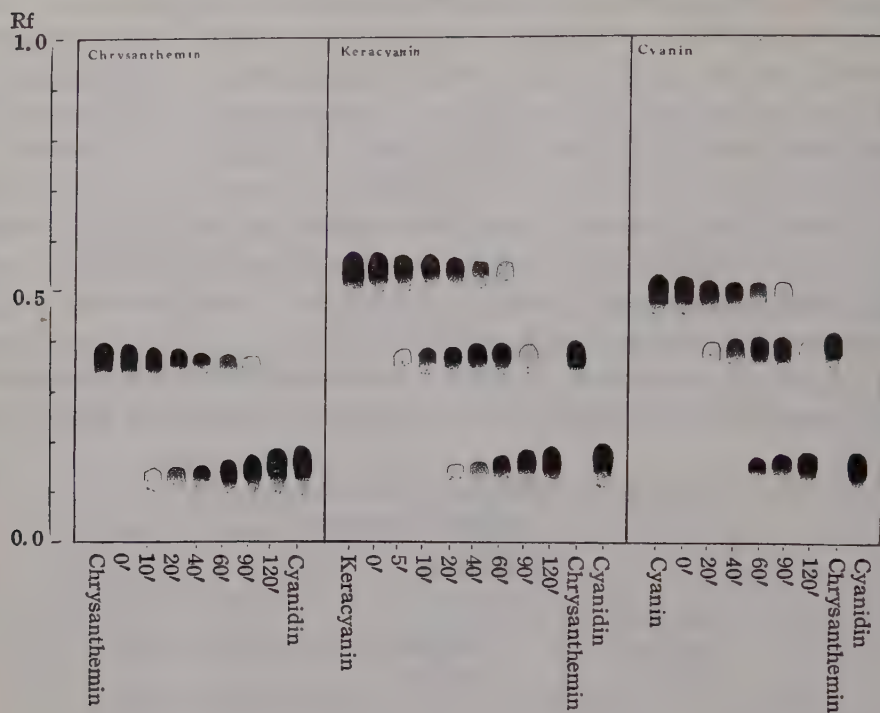
(b) Developed with solvent Bu·H
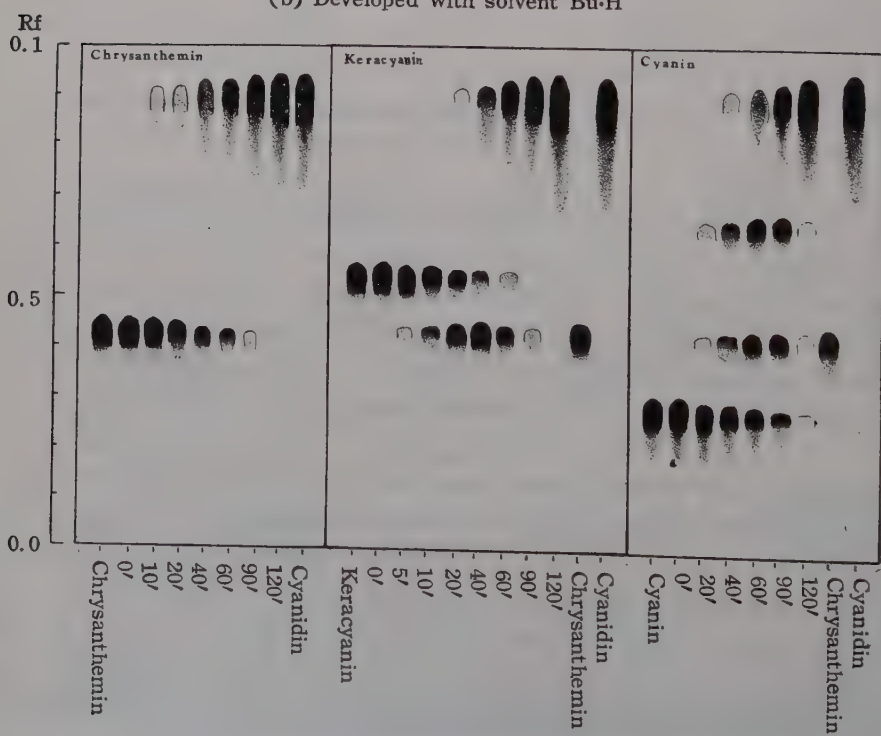
as usual. Among them, the spot No. 2 was characterized by its yellow fluorescence under ultralviolet light, while the spot No. 3 did not show any fluorescence at all. According to Willstätter (9), yellow fluorescence can be observed in 5-monoglucoside, but not in 3-monoglucoside, of pelargonidin. Therefore, the two pigment spots No. 2 and 3 in question may safely be interpreted as represented by a 5-monoside and 3-monoside, respectively.

Summary

1. In order to qualify the properties of useful solvent mixtures, paper chromatographic examinations were carried out using authentic samples of various anthocyanins. As illustrated in Fig. 1, interesting relationship was found between R_f values and pigment structures.

2. Interesting also is the fact that diglycosides are degraded stepwise into monoglucosides, when treated with warm hydrochloric acid and the resuts can be clearly demonstrated chromatographically. Thus, the glycoside type of anthocyanins is indicated by paper chromatographic examination of the products of partial hydrolysis.

Acknowledgement

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日本産タンポポ類の核型分析*

西 岡 泰 三**

Taizô Nisioka**: Karyotype Analysis in Japanese Cichorieae*

タンポポ類 (family Compositae, tribe Cichorieae) の細胞遺伝学的 研究としては先ず Babcock を中心としたクレピス属 (*Crepis*) における大きな業績をあげなくてはならない (Babcock '47). 他の属についても Babcock and Stebbins ('37) 及び Stebbins et al. ('53) によって染色体は広範囲に調査された。日本に分布するタンポポ類は大井 ('53) によると 15 属を数えるが, 各属中に含まれる種及び変種数はタンポポ属を除いてあまり多くない。これらの染色体研究は上記の研究の外に, 田原 ('10) 石川 ('21) 岡部 ('32, '51) 松浦及び須藤 ('35) 竹本 ('52, '54) などがあり, 又小野 ('43, '51, '55), 小野及び酒井 ('52, '54) による属間雑種に関する報告がある。しかし日本産タンポポ類全般についての核型分析の報告はない。著者は日本産の全属についての核型分析を続けているが, ここでは今迄得られた結果の一部を報告する。

材料及び方法

材料の植物は一植物 (リュウゼッサイ) を除いてすべて野外で採集したものである。一旦鉢植にした植物の根端を Tjio and Levan ('50) の方法を多少修正した処法で処理し染色体の観察を行った。即ち根端を 0.002 mol 8-オキシキノリン水溶液により 20°C で 2〜3 時間前処理後 0.5%醋酸オルセインとN-HCl の 9:1 混合溶液中に 60°C で 6 分間浸して, 固定, 染色, 解離を同時に行い,

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* 東京都立大学理学部生物学教室形態学講座業績第 16 号
** 東京 都立 大学 理学部 生物学 教室 Biology Department, Faculty of Science, Tokyo Metropolitan University, Tokyo, Japan.

押しつぶし法で染色体を観察した。染色体は 2000 倍に拡大しアッベ氏の描画装置を用いて描き印刷の時 2/3 に縮めた。学名は大井 ('53) に, 核型の表し方は篠遠 ('43) によった。

結 果

A コウゾリナ属 *Picris* Linn.

1. コウゾリナ *P. japonica* Thunb. (東京近郊産) Fig. 1.

2. タカネコウゾリナ var. *alpina* (Koidz.) Ohwi (長野県仙丈岳産)

後者は高山性の変種であるが, 前者との間に核型の差は見られない。 最大の染色体は ほぼ 中央に, 他は次末端部に狭窄を持ち, 仁染色体1対を持つ。核型は次の様に表わされる。

$$K(2n) = 10 = 2A^{sm} + 2tB^{st} + 2C^{st} + 2D^{st} + 2E^{st}$$

B タンポポ属 *Taraxacum* Wiggers.

3. カンサイタンポポ *T. japonicum* Koidz. (京都産) Fig. 2.

タンポポ属中での両性生殖をする種として普通のものである。二次狭窄を持つ染色体2対を含めて 16 本の染色体を持つ。核型は次の通りである。

$$K(2n) = 16 = 2^{cs}A^{sm} + 2B^{sm} + 2C_1^{sm} + 2C_2^{m} + 2D_1^{sm} + 2^{cs}D_2^{sm} + 2E_1^{sm} + 2E_2^{sm}$$

C ミヤマコウゾリナ属 *Hieracium* Linn.

4. ミヤマコウゾリナ *H. japonicum* Franch. et Sav. (長野県仙丈岳産) Fig. 3.

松浦及び須藤 ('35) は 2n = 14 を本種について報告しているが, 著者は 18 本の染色体を 観察した。附随体を持つ染色体は2対見られ, 核型は下のように示される。

$$K(2n) = 18 = 2A^m + 2B_1^{sm} + 2B_2^{sm} + 2tB_3^{sm} + 2B_4^{sm} + 2tC^{sm} + 2D_1^{sm} + 2D_2^{st} + 2E^{sm}$$

Figs. 1~10. Somatic chromosomes of Japanese Cichorieae. ×1300.

1) *Picris japonica*. 2) *Taraxacum japonicum*. 3) *Hieracium japonicum*. 4) *Hololeion Krameri*. 5) *Crepidiastrum Keiskeanum*. 6) *C. platyphyllum*. 7) *Ixeris dentata*. 8) ———— var. *amplifolia*. 9) *I. nipponica*. 10) *I. dentata* var. *alpicola*.

D スイラン属　*Hololeion* Kitam.

5. スイラン　*H. Krameri* (Franch. et Sav.) Kitam.（大阪府高槻産）　Fig. 4.

本種は東海道以西に分布している。ほとんど中央に近い狭窄を持つ染色体 16 を算する。核型は次の通りである。

$$K(2n)=16=2A^{sm}+2B_1{}^{sm}+2B_2{}^{sm}+2tC_1{}^{sm}$$
$$+2C_2{}^{sm}+2C_3{}^{sm}+2D_1{}^{sm}+2D_2{}^{sm}$$

E アゼトウナ属　*Crepidiastrum* Nakai

6. アゼトウナ　*C. Keiskeanum* (Maxim.) Nakai（愛知県知多半島産）　Fig. 5.

7. ワダン　*C. platyphyllum* (Franch. et Sav.) Kitam.（神奈川県葉山産）　Fig. 6.

両者の間に核型の差は認められないが，前者の方が常にいくらか大きい染色体を持つ。最大の染色体は長い狭窄を持ち，この部分に仁が形成される。核型は次の様に表わされる。

$$K(2n)=10=2A^{sm}+2B^{st}+2C^{st}+2D^{st}+2E^{sm}$$

F ニガナ属　*Ixeris* Cass.

8. ニガナ　*I. dentata* (Thunb.) Nakai（東京近郊産）　Fig. 7.

9. ハナニガナ　var. *amplifolia* Kitam.（東京近郊産）　Fig. 8.

10. タカネニガナ　var. *alpicola* (Takeda) Ohwi（群馬県谷川岳産）　Fig. 9.

11. イソニガナ　*I. nipponica* Nakai（新潟県柏崎産）　Fig. 10.

4 者はニガナ属中で染色体基本数 7 を持つ仲間
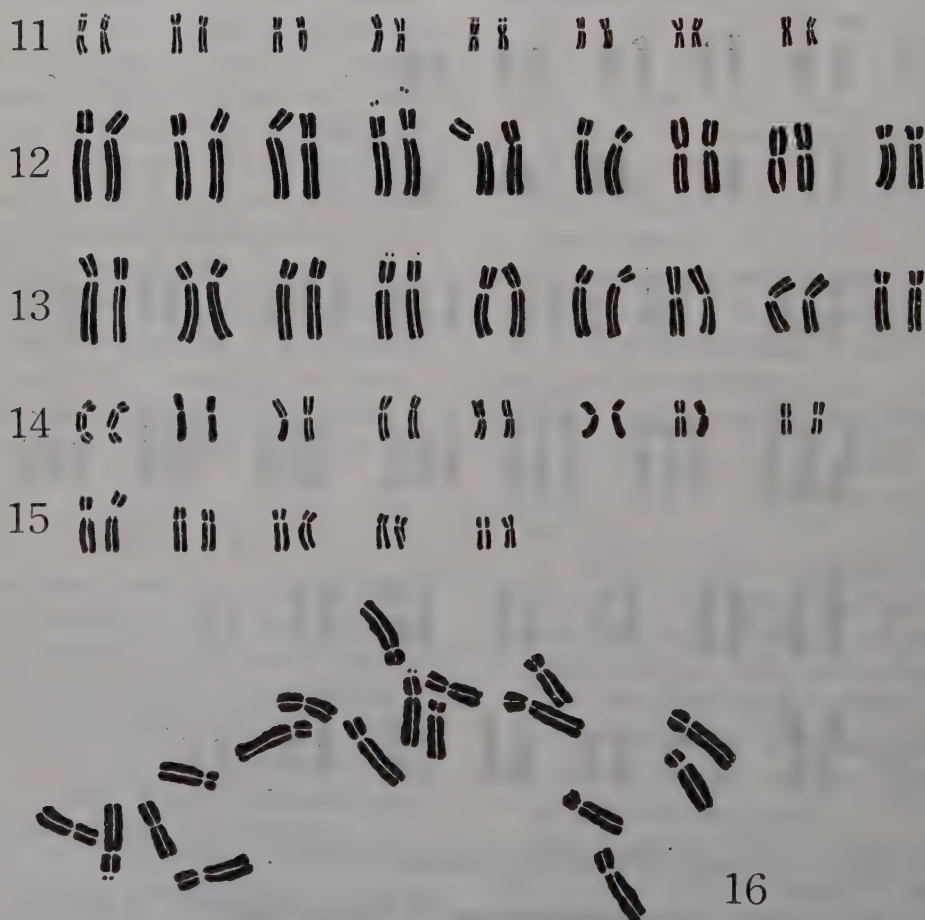
Figs. 11~16.　Somatic chromosomes of Japanese Cichorieae.　×1300.
11) *Ixeris tamagawaensis*.　12) *Lactuca indica* var. *elata*.　13) ——— var. *dracoglossa*.
14) *Youngia japonica*.　15) *Paraixeris denticulata*.　16) *Lactuca Raddeana*.

であり，高山性のタカネニガナ，海浜性のイソニ
ガナは両性生殖をする 2 倍体，ニガナ及びハナニ
ガナは単為生殖を常習とする 3 倍体である。2 倍
体植物と 3 倍体植物は核型から見てほぼ同質倍数
関係にあると思われる（竹本 '54）。

　竹本は最大で 2 狭窄を持つ染色体は，ニガナで
は 2 本，ハナニガナでは 1 本と報告して居るが，
著者は，両者において，共に 3 本を観察した。こ
れは単為生殖を常習とするため，産地によって起
って居る染色体変異が保存されるためであろう。
タカネニガナ，イソニガナの核型は大体同じで次
の様に表わせる。

$$K(2n)=14=2^{cs}Am+2Bsm+2C_1sm+4C_2sm$$
$$+2Dm+2Est$$

　最小の 1 対の染色体を除き，他はほゞ中央に狭
窄を有し，仁染色体の形には特長がある。単為生
殖植物についても大体以下の核型が成立つものと
考えられる。

$$K(2n)=21=3^{cs}Am+3Bsm+3C_1sm+6C_2sm$$
$$+3Dm+3Est$$

12. カワラニガナ　*I. tamigawiensis* (Makino)
　　Kitam.（東京都多摩川産）　Fig. 11.

　　石川（'21）の観察と同じく，小さい 16 本の染色

体が見られた。本種はニガナ属中で基本数 8 を持
つイワニガナ（*I. stolonifera* A. Gray）などの仲
間であり，核型も類似する（竹本 '52）。7 数性の
ニガナの仲間と比べて染色体の大きさが極端に小
さいのは注目に価する。2 対の二次狭窄染色体を
持って居る。核型は次の通り表わせる。

$$K(2n)=16=2^{cs}A_1m+2A_2sm+2B_1sm+2B_2sm$$
$$+2^{cs}C_1m+2C_2sm+2D_1sm+2D_2sm$$

　G アキノノゲシ属　*Lactuca* Linn.

13. アキノノゲシ　*L. indica* Linn. var. *laciniata*
　　(O. Kuntze) Hara（東京近郊産）　Fig. 12.

14. リュウゼツサイ――――――― var. *draco-
　　glossa* (Makino) Kitam.（栽培品）　Fig. 13.

15. ヤマニガナ　*L. Raddeana* Maxim. var. *elata*
　　(Hemsl.) Kitam.（神奈川県多摩丘陵産）　Fig.
　　16.

　3 者とも大体同じ核型を持つ。ほゞ中央に狭窄
を持つ小さい 2 対の染色体に特長がある。核型は
次の通り表わせる。

$$K(2n)=18=2Asm+2B_1st+2B_2sm+2tB_3sm$$
$$+2C_1sm+2C_2sm+2Dsm+2E_1sm+2E_2st$$

　H オニタビラコ属　*Youngia* Cass.

16. オニタビラコ　*Y. japonica* (Linn.) DC.（愛
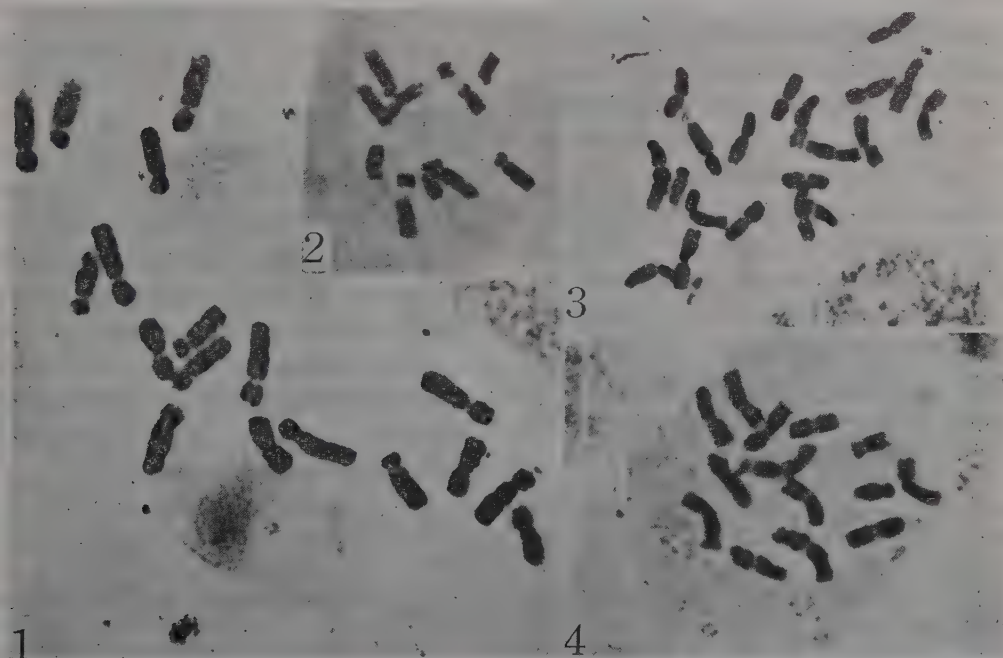
Photo. 1～4.　Somatic chromosomes of 1) *Lactuca Raddeana*, 2) *Crepidiastrum Keiskeanum*, 3) *Ixeris dentata*, 4) ――――― var. *alpicola*, ×1300.

知県篠島産） Fig. 14.

田原（'10）の報告と同様，16 の染色体を観察した。3 節構造を持つ染色体 1 対を持つ。核型は次のように表わせる。

$$K(2n) = 16 = 2csA^{sm} + 2B^{sm} + 2C_1^{sm} + 2C_2^{sm} + 2D_1^m + 2D_2^{sm} + 2E_1^{sm} + 2E_2^{sm}$$

I ヤクシソウ属　*Paraixeris* Nakai

17. ヤクシソウ　*P. denticulata* (Houttuyn) Nakai（東京近郊産）　Fig. 15.

核型は大体ワダン属と同様であるが，4 番目の大きさの染色体の狭窄の位置がわずかに異る。核型は次の式で表わされる。

$$K(2n) = 10 = 2A^{sm} + 2B^{st} + 2C^{st} + 2D^{st} + 2E^{sm}$$

論　　議

調査の対象となった種類数が少く，系統的な議論は出来ないが 2, 3 の知見をのべる。

ミヤマコウゾリナの体細胞染色体について，松浦及須藤（'35）は 14 であると報告しているが，筆者は 18 を観察した。Darlington and Janaki Ammal（'45）は松浦及須藤の報告した本種を除いて，ミヤマコウゾリナ属の染色体基本数は 9 であることを記載し，Stebbins et al.（'53）も同様な結果を認めていることからして，筆者の観察と考え合せると，前報告は誤りではないかと思われる。日本ではこの属には大井（'53）によると外にヤナギタンポポ（*H. umbellatum* Linn. var. *japonicum* Hara）のみしかないが，この植物も 2n=27 で 9 を基本数としている（西岡未発表）。したがってミヤマコウゾリナ属の基本数は 9 のみではないかと考えられる。

ニガナ属は染色体の数，形，大きさの上から 2 つの群に分けられる事を石川（'21）は花粉母細胞成熟分裂の観察の結果から主張したが，著者もこの考えを支持したい。即ち基本数 7（以下 b=7 と略書する）のニガナの群と b=8 のイワニガナの群とでは染色体の形状，数，大きさは全く異り，

前者は後者に比して染色体がはるかに大形である。この両群の関係は以後の調査で明らかにしていきたい。生活環境と分化との関聯に於て興味深いのは b=7 の群で，両性生殖を営む 2 倍体植物が特殊環境（イソニガナは海浜，タカネニガナは高山に生育）に分布しているのに，3 倍性の単為生殖を常習とするニガナやハナニガナは広く一般に分布している。この問題は生活力，繁殖力の双方からも検討されなくてはならない。なおクモマニガナ［*I. dentata* Nakai var. *Kitamurana* (Kitam.) Ohwi］は 4 倍体で単為生殖を営む高山性植物である（竹本 '54 植物学会 19 回大会報告）。

ヤクシソウ（2n=10）とワダン（2n=10）及びアゼトウナ（2n=10）との関係は自然及び人工雑種の研究（小野 '51, '55, 小野及び酒井 '54）でよく知られているが，これ等の核型はお互に極めて類似し，稔性の高い雑種ができやすいことを示しているように見える。しかし同じく属間雑種の研究された（小野 '43, 小野及酒井 '51）ヤクシソウ（2n=10）とアキノノゲシ（2n=18）とでは核型は全く異り，この間の雑種形成は全く特異的といわなければならない。この雑種の体細胞染色体数は期待される様に両親の和の 14 にはならないで，10～14 のいろいろな数の染色体を持つ細胞がキメラになって存在し，その内 12 のものが最も安定なものとなる。この場合の染色体の減少は母親方の細胞質の影響で，主に父親方で起っていると考えられ，この様な現象は伊豆に於けるアゼトウナの自然雑種集団の中でも起っていると考えられる（小野及び酒井 '54）。雑種形成のさい新しく出来た染色体組の平衡の問題は今後に残されている。これ等のタンポポ類に特有な雑種形成や染色体変異を追究するための基礎的な知識として核型の分析が有意義である。

終に当り本研究を指導された小野記彦教授ならびに材料（イソニガナ）を提供して頂いた東北大学理学部岡部作一氏に深く感謝する。

Summary

1) The karyotypes of 13 species and 4 varieties of Japanese Cichorieae are reported.

The results are as follows:

1. *Picris japonica* Thunb.

2. ——— var. *alpina* (Koidz.) Ohwi

$K(2n)=10=2A^m+2^tB^{st}+2C^{st}+2D^{st}+2E^{st}$

3. *Taraxacum japonicum* Koidz.

$K(2n)=16=2^{cs}A^{sm}+2B^{sm}+2C_1^{sm}+2C_2^m+2D_1^{sm}+2^{cs}D_2^{sm}+2E_1^{sm}+2E_2^{sm}$

4. *Hieracium japonicum* Franch. et Sav.

$K(2n)=18=2A^m+2B_1^{sm}+2B_2^{sm}+2^tB_3^{sm}+2B_4^{sm}+2^tC^{sm}+2D_1^{sm}+2D_2^{st}+2E^{sm}$

5. *Hololeion Krameri* (Franch. et Sav.) Kitam.

$K(2n)=16=2A^{sm}+2B_1^{sm}+2B_2^{sm}+2^tC_1^{sm}+2C_2^{sm}+2C_3^{sm}+2D_1^m+2D_2^{sm}$

6. *Crepidiastrum Keiskeanum* (Maxim.) Nakai

7. *C. platyphyllum* (Franch. et Sav.) Kitam.

$K(2n)=10=2A^{sm}+2B^{st}+2C^{st}+2D^{st}+2E^{sm}$

8. *Ixeris dentata* (Thunb.) Nakai

9. ——— var. *amplifolia* Kitam.

$K(2n)=21=3^{cs}A^m+3B^{sm}+3C_1^{sm}+6C_2^{sm}+3D^m+3E^{st}$

10. ——— var. *alpicola* (Takeda) Ohwi

11. *I. nipponica* Nakai

$K(2n)=14=2^{cs}A^m+2B^{sm}+2C_1^{sm}+4C_2^{sm}+2D^m+2E^{st}$

12. *I. tamagawaensis* (Makino) Kitam.

$K(2n)=16=2^{cs}A_1^m+2A_2^{sm}+2B_1^{sm}+2B_2^{sm}+2^{cs}C_1^m+2C_2^{sm}+2D_1^{sm}+2D_2^{sm}$

13. *Lactuca indica* Linn. var. *laciniata* (O. Kuntze) Hara

14. ——— var. *dracoglossa* (Makino) Kitam.

15. *L. Raddeana* Maxim. var. *elata* (Hemsl.) Kitam.

$K(2n)=18=2A^{sm}+2B_1^{st}+2B_2^{sm}+2^tB_3^{sm}+2C_1^{sm}+2C_2^{st}+2D^{sm}+2E_1^{sm}+2E_2^{st}$

16. *Youngia japonica* (Linn.) D. C.

$K(2n)=16=2^{cs}A^{sm}+2B^{sm}+2C_1^{ms}+2C_2^{sm}+2D_1^m+2D_2^{sm}+2E^{sm}+2D_2^{sm}$

17. *Paraixeris denticulata* (Houttuyn) Nakai

$K(2n)=10=2A^{sm}+2B^{st}+2C^{st}+2D^{st}+2E^{sm}$

　　2) Eighteen chromosomes are observed in *Hieracium japonicum*, while fourteen chromosomes were reported previously in this species by Matsuura and Sutô (1935).

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セイタカタンポポ×ヒロハタンポポにおける
不 和 合 性 遺 傳 子 の 性 質

タンポポの自家不和合性に関する研究 I

岡 部 作 一[*]

Sakuichi OKABE[*]: On the Nature of Incompatibility Alleles in *Taraxacum elatum* × *T. longe-appendiculatum*
(Self-incompatibility Studies in *Taraxacum* I)

East (1940) によると菊科植物 1400 種のうちその約 25% は自家不稔であるという。そのうちキクニガナ (Stout 1916) やコスモス (Little, Kantor and Robinson 1940) についての遺伝学的研究が行われているが，これらの植物の不和合性は複雑で充分な説明が与えられていない。ところが Gerstel (1950) の行った *Parthenium argentatum* の自家不和合性に関する研究は非常にあざやかで従来困難とされていた菊科植物の不和合性の遺伝研究に新しい道をひらいたものである。彼は各複対立遺伝子間には幾段階かの強弱の差があり，それにより遺伝子相互の間に優劣関係の生ずること，及び花粉は自身の遺伝子よりも母細胞の遺伝子型に支配されること即ち造胞体反応 (sporophytic reaction) の現象を明かにして複雑な和合関係を困難なくきれいに説明することができた。同年 Hughes と Babcock も *Crepis foetida* subsp. *Rhoeadifolia* の自家不和合性が *Parthenium* と同じ機構によるものであることを発表した。

タンポポ (*Taraxacum*) も菊科の一員で二倍性の種類が自家不稔であることは古くから一般に知られていた。しかしその遺伝様式については何も明かにされておらない。筆者の本邦産のタンポポを材料とした研究は未だその途中にあるものであ

るが今日までの結果を報告する。自家不和合性の研究においては材料相互間の総当り式交雑を行う必要上同一遺伝質を持った個体を沢山用意しなければならぬ。タンポポはその点好都合にも再生力が強いので根の cutting によつて交雑の規模に応じて必要な鉢数を用意することができる。また多年生であるため F_1 以後の子孫と母体との交雑も容易に行うことができる。交雑するには先ず開花 2～3 日前の蕾にパラフィン紙製の袋をかけておき，開花と共に袋をはずし二つの頭状花をお互に接触して相互受粉を行う。Gerstel や Hughes は受粉 1～2 時間後に柱頭上の花粉の発芽を調べて和合・不和合を判定したが筆者の場合は交雑後再び袋掛をしておき後に実際にみのった果実数を数えた。同じ組合せの交雑を数回行い，その平均着果数を表にあらわした。

種内及び種間交雑試験

自家不和合性の植物は別な株との間では交雑不和合のこともあるが一般には強い交雑和合性を示すことが多い。離反遺伝子説によると両植物共同一の遺伝子を持つものであれば自家受粉の場合と同様に両者は和合しないが，若し $S_1S_2 × S_1S_3$ のように少くとも共通でない遺伝子が一組ある時は和合することになる。日本各地産のタンポポ 5 種の種内及び種間交雑の結果は第 1 表に示された通りであった。着果数は最少 3 頭状花の平均で実験数が少くあまり結果のはっきりしない組合せもあるが T_1 と T_7 及び Y_1 と O_3 をのぞけば他の大部

* 東北大学理学部生物学教室 Biological Institute, Faculty of Science, Tōhoku University, Sendai, Japan.

Table 1. Intra- and interspecific crossings of five *Taraxaca*. Mean nos. of filled achenes in this table were based on results from at least three heads. +is fertile, But failed to count.

Species	Locality and plant nos.	♂ / ♀	F_1	K_1	M_1	M_2	Y_1	Y_2	N_1	H_1	O_1	O_2	O_3	S_1	T_1	T_5	T_6	T_7	C_1
T. japonicum Koidz. カンサイタンポポ	Fukuoka	F_1	–	30	15	49	43	16	21	20	27	45	56	14	30	44	27	48	16
	Kyōto	K_1	7	–	7	8	7	5	12	6	6	7	6	4	5	9	5	11	5
T. elatum Kitam. セイタカタンポポ	Sakashita	M_1	45	25	–	40	60	56	61	58	44	29	29	61	35	47	46	18	76
	"	M_2	51	17	41	–	57	50	4	33	5	36	68	74	53	80	63	78	32
T. longe-appendiculatum Nakai ヒロハタンポポ	Yamada	Y_1	25	3	13	10	–	11	11	12	9	24	0	8	31	18	15	6	16
	"	Y_2	11	6	17	5	17	–	14	21	10	23	35	31	6	19	36	26	25
	Nagoya	N_1	53	28	31	23	38	47	–	28	61	27	51	21	39	57	1	49	34
	Shimoda	H_1	24	3	45	19	54	37	62	–	43	34	27	4	33	45	17	44	44
	Ōshima	O_1	55	29	20	29	38	44	43	32	–	37	20	28	52	72	30	30	59
	"	O_2	39	37	56	23	22	39	23	16	38	–	48	55	32	14	40	47	2
	"	O_3	57	26	23	+	1	30	39	32	35	33	–	21	29	44	91	49	56
T. sendaicum Kitam. センダイタンポポ	Sendai	S_1	53	14	42	48	40	35	34	35	48	35	39	–	37	53	41	36	53
T. platycarpum Dhlst. カントウタンポポ	Tōkyō	T_1	19	11	43	1	48	30	59	25	47	54	54	16	–	18	61	0	49
	"	T_5	70	78	88	82	19	66	93	64	70	114	62	53	76	–	86	57	+
	"	T_6	35	14	43	59	33	67	16	0	11	63	78	8	+	54	–	101	45
	"	T_7	27	5	11	22	7	38	9	9	20	23	17	24	4	33	19	–	15
	Chiba	C_1	24	24	+	32	55	77	79	61	87	11	61	81	66	129	60	78	+

分の組合せは交雑和合であるとみてよかろうと思う。タンポポには後に述べるように遺伝子間の強弱の差と造胞体反応があるため $S_1S_2 \times S_1S_3$ が和合しないような場合も出てくる。この試験結果により日本のタンポポには互に複対関係をなす多数の離反遺伝子が分布していることが結論される。Gerstel の *Parthenium argentatum* では 14 組合せのうちその 12 までがよく和合した。菊科以外の植物では *Oenothera organensis* に 35 (Emerson 1939)、アカツメクサに 41 (Williams 1939) の S遺伝子が知られている。タンポポにおけるこれら S遺伝子の性質を知るためにそのうちの一つの組合せ $M_1 \times N_1$ の子孫の遺伝子分析を行ってみた。

セイタカタンポポ×ヒロハタンポポにおける不和合性遺伝子の分析

セイタカタンポポ (*T. elatum* Kitamura) は京都大学の北村四郎教授から送られたもので、美濃の坂下産、ヒロハタンポポ (*T. longe-appendiculatum* Nakai) は筆者が名古屋市郊外で採集した材料でこの両者はよく和合する。その F1 12 個体をとり、それに両親を加え全体 14 個体の間で相互交雑を行った。その平均着果数を示したのが第 2 表で、和合関係を図解したのが第 4 図である。F1 が A, B, C, D の 4 組に分れたことによって両親には共通の遺伝子が一つもなく S_1S_2、及び S_3S_4 の如き構成のものであったことが明かであ
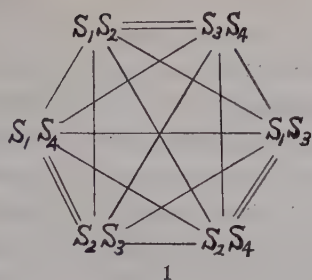
Table 2. Compatibilities and incompatibilities in crosses between sibs ex *Taraxacum elatum* × *T. longe-appendiculatum* and their parents. Each count represents mean no. of filled achenes that of at least five heads pollinated.

♀ Genotype of sporophyte / ♂ Phenotype of pollen grains	(P) S_3	(P) S_1S_2	(F1) A S_1				B S_2S_3					C S_2	D S_1S_3	
Plant nos. ↓	M_1	N_1	M_1N_1 -2	″ -6	″ -8	″ -12	″ -4	″ -5	″ -7	″ -9	″ -10	″ -1	″ -3	″ -11
(P) S_3S_4　M_1	—	28	7	49	31	47	7	7	1	1	0	33	1	2
(P) S_1S_2　N_1	61	—	0	0	2	1	9	1	0	9	4	1	1	1
(F1) A S_1S_4　M_1N_1 -2	16	0	—	0	0	1	31	28	24	25	35	37	0	0
″ -6	58	0	1	—	0	1	38	43	35	46	37	11	0	0
″ -8	45	0	0	0	—	0	17	45	15	44	33	1	6	0
″ -12	80	0	1	2	1	—	52	50	54	38	38	11	0	0
B S_2S_3　″ -4	1	9	22	40	28	45	—	0	0	0	0	2	3	4
″ -5	1	1	23	57	55	49	0	—	0	0	0	1	0	1
″ -7	0	1	12	30	13	23	0	0	—	0	0	0	0	0
″ -9	6	8	36	36	50	58	2	1	0	—	0	0	6	3
″ -10	1	3	58	35	39	32	0	0	0	1	—	1	2	0
C S_2S_4　″ -1	16	1	22	21	15	12	3	0	0	0	0	—	10	18
D S_1S_3　″ -3	4	0	0	0	2	0	0	2	4	2	3	13	—	2
″ -11	2	0	0	0	1	0	1	17	0	2	4	26	0	—

<div style="display:flex">
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る。これら6組間の 15 組合せのうち和
合組合せは只6つだけで他の9組合せは
不和合であった。こんなに沢山の不和合
組合せの現われる例は今日まで他の植物
では知られておらない。この原因はこ
れに関する複対立離反遺伝子 (opposi-
tional multiple alleles) S_1, S_2, S_3, S_4
の性質にあるので, Gerstel の説に従い
S_4 は劣性であるが他の 3 遺伝子は共に
優性で造胞体反応の力があり花粉の表現
型を調整する（第 2 表上段のように）と
仮定すれば容易に説明することができ
る。なお第 2 表に示されているように
不和合組合せにおいてかなり高い稔性
を示し, 或は逆に和合組合せであると思
われる組合せに非常に稔性の低い場合な
どが現われてくる。このような偽稔性
(pseudofertility) は他の自家不和合性植
物にもしばしば現われる現象であるが,
その原因は種々で場合によって異なる。
受粉, 受精又は胚発生の時の環境の影響,
細胞質の一時的変化, 或はもっと根本的
な S 遺伝子の突然変異などが考えられ
る。この現象については将来くわしい実
験を行ってみる必要がある。

考　察

　自家不和合性は多くの植物で見られる
現象であるが, ここでは生殖器官に長短
花柱 (heterostyly) のような構造的差
異のない homomorphic (non-hetero-
styled) の場合だけについて考えること
とする。遺伝学的に最もくわしく研究さ
れているのはタバコ属, ツクバネアサガ
オ等のナス科植物で East 其他多くの学
者により S 系列による複対立離反遺伝子
説が確立されるに至った。第1図はその
一例としてハナタバコの和合関係を表し
た所謂自家不和合性の6角形である。二
重線で連絡されている組合せには同一の
離反遺伝子は一つもなく, その F_1 には
両親の型をのぞいた他の4型が現われて
くる。一重線でつながれる12組合せ (3

</div>
<div>

Figs. 1~4.

Nicotiana Sanderae
$S_1, \ S_2, \ S_3, \ S_4$
－　－　－　－
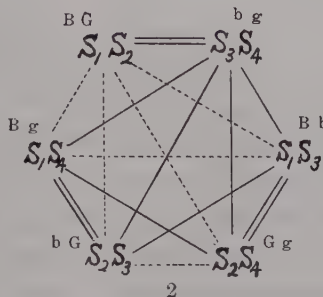
Cardamine pratensis
B　G　b　g　(Correns)
$S_1 = S_2 > S_3, \ S_4$
＋　＋　－　－
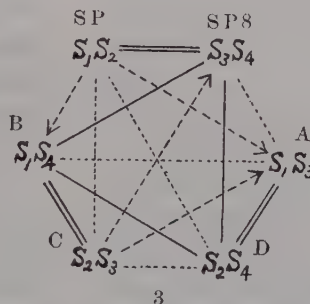
Parthenium argentatum
$R_2 > R_1, R_3 > R_4$ (Gerstel)
$S_2 > S_1 = S_3 > S_4$
＋＋　＋　＋　－
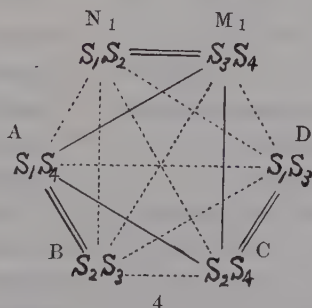
Taraxacum elatum ×
T. longe-appendiculatum
$S_1 = S_2 = S_3 > S_4$
＋　＋　＋　－

Figs. 1~4. Hexagonal diagrams of cross-
compatibilities and incompatibilities in 1)
Nicotiana Sanderae, 2) *Cardamine pratensis*
(based on Correns 1912), 3) *Parthenium
argentatum* (based on Gerstel 1950) and 4)
Taraxacum elatum × *T. longe-appendiculatum*.
＝＝ absolutely compatible, —— compatible
in two directions, ·······▸ compatible in one
direction, ······ incompatible. －weak, ＋strong,
＋＋more stronger alleles.

</div>
</div>

個の直角三角形と 1 個の正三角形の辺にあたる）においては花粉の半分すなわち柱頭と共通の遺伝子を有する花粉は和合することができないためにその次代は 2 種類で母親の型は現われない。ここで一番重要なことは花粉粒は環元分裂によって分配された自身の遺伝子の作用だけをあらわすのであって，その母細胞である二倍体の持っている遺伝子の影響を全く受けないことで，この現象は haploid (gametophytic) pollen control と呼ばれている。

Correns (1912) のハナタネツケバナ (*Cardamine pratensis* L. の研究は古いものであるが，その遺伝子の作用はナス科のように単純なものでなく今日から見ても重要な研究である。Correns は Bb, Gg で示される花粉形成に際して優劣関係を有する 2 組の対立遺伝子でこれを説明した。彼の説によると Bb×Gg の F_1 には BG, Bg, bG, bg の 4 型が生じ，共通の優性遺伝子を有する組合せは和合しない。花粉形成の時には造胞体反応を起し，優性遺伝子は相互に影響し合い，若し劣性遺伝子のある時はそれを支配する。ここで bg だけが他のすべての組と和合するのは bg では *Nicotiana* の場合と同様に haploid pollen control だけが行われるからである。この和合関係は第 2 図に示されたように今日の複対立離反遺伝子説によっても何の不都合もなく説明し得られるものである。只第 1 図のナス科の場合とのちがいはハナタネツケバナでは S_1S_2 と S_3S_4 との間に強さの差があり，S_1 と S_2 は造胞体反応，即ち diploid control の力を持っていることである。

第 3 図は Gerstel の *Parthenium argentatum* の説明図で彼の Fig. 1 を説明し易い形に直したものである。一見非常に複雑しているように見えるが原理は前の *Cardamine* の場合と同様で，只遺伝子の強さが 2 段階でなくここではそれが $S_2 > S_1 = S_3 > S_4$ のように 3 段階になっているためにこうなるのである。S_2 が最強で S_1, S_3, S_4 に対して支配力を有し，S_4 は逆に最も弱く S_1, S_2, S_3 の支配をうける。S_1 と S_3 はその中間で同格とも云うべく相互に影響し合うばかりでなく S_2 には弱く S_4 を支配する。それ等の関係で一方向的和合が 4 ケ処に起っている。例えば $S_1S_4 × S_1S_2$ では S_2 が S_1 に対して優性であるため花粉は全部 S_2

の表現型を示し S_1S_4 柱頭上で発芽し得るが逆の $S_1S_2 × S_1S_4$ では今度は S_1 が優性となるため花粉は全部 S_1 の作用を現わし S_1S_2 と和合することができなくなる。

Hughes と Babcock の *Crepis foetida* subsp. *Rhoeadifolia* の不和合性の遺伝も *Parthenium* と非常によく似ており，遺伝子の強弱関係は $S_1 < S_2 < S_3 = S_4$ の 3 段階に分れ，$S_1S_2 × S_2S_3$ 及び $S_1S_2 × S_2S_4$ は和合するがその逆方向へは和合しない。

さて第 4 図はタンポポ $M_1 × N_1$ 及びその F_1 の和合関係を示したものであるが，*Parthenium* や *Crepis* よりも Correns の *Cardamine* に近い。ここでは S_4 だけが弱性であるため和合組合せが *Cardamine* よりも 3 組合せだけ少くなり，逆に不和合組合せは 3 組合せ多くなっている。弱性遺伝子一つを共通に持つ組合せ例えば $S_1S_4 × S_3S_4$ から生ずる次代に S_4S_4 のような同型接合体が全体の 25% 現れることはいずれの場合にも共通した現象である。

第 2 図から第 4 図までに示された例はその和合関係の細部にわたっては優性遺伝子の数とそれらの間の強さの段階とにより幾分の差をあらわしているが，Babcock と Hughes (1950) があげているようにみな次の 4 項目の原理に支配されている。即ち 1) one gene with multiple alleles, 2) independent gene action in the pistil, 3) diploid (sporophytic) pollen control, 4) dominance in the anthers がその 4 項目で，はじめの 2 項目は *Nicotiana* 型と共通の性質である。Gerstel や Babcock 達はこの型を *Crepis-Parthenium* 型或は菊科型と呼んでいるが，アブラナ科には *Cardamine* ばかりでなく，タマナ（柿崎 1930），*Capsella grandiflora* (Rily 1936), ダイコン（建部 1944）等にこの型の不和合性のあることが古くから知られており，叉最近 Bateman (1955) も *Iberis amara* 並に其他のアブラナ科植物でこれを確認したという。菊科植物ではその不和合性の研究されたものの数はアブラナ科植物などにくらべてはるかに少い。菊科が皆この型に属するかどうかはもっと沢山の種類について研究を進めてからでないとわからない。若しこの型を今菊科型と呼ぶ位ならばむしろ *Cardamine* 型と云って置いた方がよいのではないかと思う。

Summary

1. Crosses were made between 17 self-incompatible plants belonging to five diploid species of *Taraxacum*. The existance of a large number of incompatibility alleles in genus *Taraxacum* is suggested by the occurrence of few cross-incompatible combinations (Table 1).

2. Progenies of a cross between two self-incompatible species, *T. elatum* Kitamura and *T. longe-appendiculatum* Nakai were genetically analysed and found to consist of four intra-incompatible classes (Table 2). The compatibilities and incompatibilities among these four classes and two original parental plants can be explained by a hexagonal diagram on the assumption that a single series of four oppositional multiple alleles is responsible (Fig. 4).

3. Pollen behavior is sporophytically controlled. All the pollen grains from one plant act alike and depend on the genotype of its parental sporophyte. In this experiment S_4 is recessive to each of the other three alleles. S_1, S_2 and S_3 are "Strong" and dominant over S_4, but each strong allele exhibits equal dominance values in the presence of the other. Both S_1 and S_2 pollen grains from a mother cell with $S_1 S_2$ alleles behave equally as $S_1 S_2$. Therefore, plants having one strong allele in common are cross-incompatible.

4. Dominance is not expressed in the pistil.

5. The self-incompatibility system found in *Taraxacum* is of the same type as that described for *Crepis-Parthenium*.

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伊豆諸島の植物分布について

鈴　木　　泰*

Tai SUZUKI*: On the Plant Distribution over Izu-Islands

　関東南方海上，大体北緯 32° の青ケ島から 35° の大島まで，9つの島がほぼ南北に並んでいる。この島々に，日本として北の方に分布する植物―北方系―と，南の方に生育する植物―南方系―がどの様に分布しているか，その限界はどの辺にあるか，それを探求するのが本研究の主目的である。但しこれはどこまでも伊豆諸島のみに関してであって，内地及緯度の関係については全然考えない。1949 年以来，毎夏，いずれかの島に渡り先ず各島の植物採集を行った。其の結果「伊豆諸島植物分布目録」を 1956 年 2 月にまとめ上げた。この目録作成の資料は，東京都建設局観光課編「伊豆七島の植物」―非売品―（常谷幸雄氏が多年にわたり各誌に報告されたものを集録編成したもの）を基礎として，資源研，水島正美氏が追加した大島植物目録，お茶の水大津山尚氏が追加し訂正した三宅島植物目録，科博佐竹義輔氏が訂正し追加した八丈島植物目録，水島正美氏の青ケ島調査報告（筆者未渡島），筆者自身が採集し作成した利島新島式根島神津島御蔵島植物目録等である。

　各島別及び植物群別の植物の種数は下表の如くである。

　目録中から明らかに畠作の栽培植物と思われるものは全部除いたが，若干の移植植物及帰化植物が含まれている。此の中，南方系の植物としてマークしたもの 103 種，北方系の植物としてマーク

全島総記載数

植　　物	科 数	種 数
しだ植物	14	131
裸子植物	7	13
双子葉植物 原生花被類	67	367
双子葉植物 後生花被類	31	240
単子葉植物	19	287
合　　計	138	1038

島名／植物分類	大島		利島		新島		式根島		神津島		三宅島		御蔵島		八丈島		青ケ島	
	科	種	科	種	科	種	科	種	科	種	科	種	科	種	科	種	科	種
しだ植物	6	46	10	44	10	40	3	18	11	57	12	56	10	52	14	81	7	37
裸子植物	7	11	5	7	4	7	3	3	4	4	2	5	2	4	4	6	5	5
双子葉原生花被類	57	230	48	122	52	133	39	79	55	149	55	192	53	116	54	169	38	82
双子葉後生花被類	25	126	22	64	21	68	17	41	24	84	25	124	22	85	24	111	16	61
単子葉植物	10	134	10	73	14	82	8	39	14	117	15	141	13	77	13	123	9	40
合　計	105	557	95	314	101	330	70	180	108	414	111	518	100	332	109	490	75	225

* 東京都台東区立清島小学校　Kiyoshima Primary School, Taito-ku, Tokyo, Japan.

したもの 43 種がある。その分布数は別表 I の如くである。

別　表　I

系　統 島　名	南 方 系	北 方 系
大　　　　島	37種	5種
利　　　　島	8	0
新　　　　島	10	0
式　根　島	2	0
神　津　島	11	4
三　宅　島	11	10
御　蔵　島	10	10
八　丈　島	11	12
青　ケ　島	3	2
計	103	43

境 界 に つ い て

　館脇博士によれば，私が対照としている伊豆諸島は，東亜温帯植物区系域中の本州区系区に属していて，問題とする所は何も無い。然し，この大きな見方によればそれに違いないと思うが，植物の S 性（南方系）と N 性（北方系）について少しく拡大して見れば，伊豆諸島の何処かに一応の境界がある様に思われる。表に示した如く，南方系 103 種，北方系 43 種をマークして目録中から抽出したのであるが，問題はこのマークにある。S 性と N 性が両端に於いてははっきりするが，その中間に於いては個人の判断により違ってくることが考えられる。そこで，マークした南方系と北方系の植物から更にその分布性が顕著と認められるものを次の如く抽出する。

　南方系　ヘゴ，リュウビンタイ，タニワタリ，
　　　スジヒトツバ，カギカズラ，ハドノキ，以上
　　　6 種
　北方系　ウメガサソウ，ウメバチソウ，イワガ
　　　ラミ，コイワザクラ，マイヅルソウ，ノギラ
　　　ン，ヤマブキショウマ，以上 7 種
　この北上又は南下の状態を表示すると別表 II の如くなる。先ず南方系から検討して見る。スジヒトツバは八丈島と神津島とに在り，御蔵島と三宅

別　表　II

系統 島名	南 方 系 北　上	北 方 系 南　下
大　　島		ヤマブキショウマ ノギラン
利　　島		
新　　島		ノギラン
式根島		
神津島	スジヒトツバ	ノギラン ウメバチソウ
三宅島	リュウビンタイ タニワタリ カギカズラ	ノギラン ウメバチソウ ヤマブキショウマ ウメガサソウ
御蔵島	リュウビンタイ タニワタリ カギカズラ	ノギラン ウメバチソウ ヤマブキショウマ マイヅルソウ コイワザクラ イワガラミ
八丈島	ヘゴ リュウビンタイ タニワタリ スジヒトツバ	ノギラン ヤマブキショウマ
青ケ島	タニワタリ ハドノキ	

島にはない―或は未発見なのかも知れない―八丈島には東山水源地附近に相当豊富に生育しているが，神津島では天上山方面の一部にしか存在しない上，個体数も指を折る程度しか無い。あるという名ばかりであるが然し確かに存在する。タニワタリは青ケ島から三宅島まで，順次北上している。リュウビンタイは八丈島から御蔵島，三宅島まで北上して神津島以北の島には無い。カギカズラは御蔵島と三宅島とに生育している。以上の点から南方系植物の北上限界が大体において三宅島に在るといっても差支えないのではあるまいか。

　次に北方系植物について考えてみる。ヤマブキショウマ，ノギランは大島から八丈島まで広い分布を示している。然し御蔵島は上記二品の外にウメバチソウ，マイヅルソウ，コイワザクラ，イワガラミが生育している。これは高さ 851 m の御山山頂を中心にして附近の湿原性の所に多く生えているのである。この他この湿原中にハコネコメツツジやハクサンオミナエシの変種であるシマキンレイカ等が発育している状態を見ると，内地の高山に登った様な感じで，南へはここまでという感が強くなる。北方系植物として抽出したのは主に山地性植物になるが，南下の主要な限界を御蔵島

とするのが適当であると筆者は考えている。限界
については上述の如く，南方系植物の北上限界を
三宅島とし，北方系植物の南下限界を御蔵島とし
たい。

地 学 的 側 面 観

　上記の様に限界を一応想定したが，これを裏づ
けるものは何であろうか。

　第一は地学的考察である。東京都公園観光課編
「伊豆七島噴火史並びに地形地質」―理学博士大森
房吉氏調査報告―及び水産庁の渡辺水路部長の談
話を拠り所として考えて見る。伊豆諸島として対
象となった 9 の島の中，古く噴火を終り，噴火の記
録が全然無いのは，利島と御蔵島の両島だけであ
る。従って両島は割合安定した状態で植物が生育
しているわけである。たゞ急峻な火山島であるだ
けに風水害による生育の阻害も考えられるが，噴
火の時の熔岩流がなだれて植物を下敷にする大損
害とはくらべものにならない。特に山頂附近に生
育の場を持つ北方系植物については此の問題は大
きい。島々を採集して見てわかることは，種が一
様に散在している場合と，ポツリ一ケ所にだけあ
る場合とが相半ばしていることである。従って何
かの機会にその箇所が害を受けると，そこにあっ
た種がその島では簡単に絶滅することになる。御
蔵島御山の場合は古く噴火が終った為，その後生
育した植物が温存され，残存したものと考えてよ
かろう。

　第二に考察されるのは黒潮暖流である。黒潮は
本州南東海岸沿いに北東に流れているが，その幅
が広くなったり，狭くなったりし，その北縁部は
常に動揺する由である。大体八丈島は常に黒潮本
流中に浸されているが，御蔵島，三宅島，神津島
は北縁部附近に当り，或る時は本流中に，又或る
時は太い支流中にと常に変動していて，定型がな
いそうである。黒潮の細い支流は時に東京湾内ま
で流れ込むことがあるそうである。以上の如く御
蔵島，三宅島附近に黒潮本流の縁部があるという
ことから，この二島が最もその影響を強くうける
であろうということは肯定できる。従って御蔵島
から約 16 粁ばかり北にある三宅島が南方系植物
の北限であると同時にこの黒潮の持つ暖かさが御
蔵島に於て北方系植物の南下を止めたと考えられ
る。

　以上の 2 考察の中，黒潮暖流の影響が強く伊豆
諸島に於けるS性及N性植物の限界を左右する
と想定するので，変動する黒潮の北縁部にはさま
れた帯を黒潮線 Black Current Line と呼びた
いと思う。

結　　び

　以上伊豆諸島の植物分布の境界について論じた
が，筆者の意図する所はむしろ問題の提起であっ
て，今後これに関心を持たれる方々によって更に
検討が重ねられることを祈ってやまない。

Summary

　On the sea to the south of Kanto District, from the north to the south, Izu-
Islands consisting of Oshima, Toshima, Niijima, Shikinejima, Kozushima, Miyake-
jima, Mikurajima, Hachijoshima, and Aogashima, lie in sequence covering three
degrees in the latitude. For several years, the author has collected seed plants and
ferns distributed over these isles, and made a list of them. He notices that the
northern elements (plants growing in the northern part or mountainous region of
Japan) spread to Mikurajima, while the southern elements (plants in the southern
part of Japan or in the subtropical and tropical regions) come up to Miyakejima.
This fact is probably attributed to the Black Current in the Pacific Ocean. Accord-
ing to a report, the width of the Current is frequently changeable, so that a zone

put between the northern margins of fluctuated Current alters its width from ten to several hundreds kilometers, and the both isles of Miyakejima and Mikurajima are placed in this zone. The author supposes that the floral elements of these two isles were intensely influenced by the changeable Current, in other words, the northern elements reached Mikurajima when the Current became minimum, the southern elements arrived at Miyakejima when the Current became maximum width, and inhabited there respectively in a long past age. Thus, he would suggest to call this zone between the northern margins of the Current "Black Current Line", as far as the flora of the Izu-Islands concerned.

<div align="center">参　　考　　文　　献</div>

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本　会　記　事

支　部　通　信

北海道支部

11 月例会 (11 月 24 日，於北大農) 田川 隆: アメリカの大学.

関東支部

10 月例会 (10 月 14 日，堀 正一氏案内) 赤城山の群落の観察及び採集.

11 月例会 (11 月 17 日，於東大理植物，講義室) 高橋基生: 根系呼吸に及ぼす銅イオンの作用と其の燐酸及び珪酸との関連性並びに鉱害対策. 新崎盛敏: カサノリ類の体形成について――カサノリ属のカサ形成.

近畿支部

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九州支部

第 42 回例会 (11 月 24 日，於九大理，第 1 講義室) 佐原幸雄: 冷凍乾燥の基礎研究. 大内 準: 英彦山を中心とする植物分布.

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